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Graduate Program in Kinesiology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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Abstract

Professional breath-hold diving has been linked to acute dangers; however, the long-term impacts on vascular health are unknown. The endothelium releases vasodilator substances and its functionality is an indicator of vascular health. We are testing the hypothesis that chronic exposure to severe hypoxia may alter endothelial function in breath hold divers. Divers and controls completed a flow-mediated dilation (FMD) protocol, where brachial blood flow velocity was measured following blood flow occlusion. Percent FMD was calculated as the primary measure of endothelial function. T-tests assessed the statistical significance of between-group differences. The %FMD were similar between groups ($p > 0.05$); however, divers had reduced peak reactive hyperemia (PRH) compared to controls ($p < 0.05$). Accordingly, endothelial function was not altered in divers but they displayed reductions in PRH. This suggests that divers expressed a reduced microvascular dilatory response to ischemia, rather than impairments in endothelial function or the conduit artery response.

Keywords

Endothelial function; peak reactive hyperemia; microvasculature; breath-hold diving.

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List of Abbreviations

AIDA The Association Internationale pour le Dévélopement de l'Apnée

ATP Adenosine triphosphate

AUC Area under the curve

BA Brachial artery

BF Blood flow

BHD Breath-hold-diving

BL Baseline

BP Blood pressure

CI Confidence intervals

CMAS World Confederation of Underwater Activities

CO_2 Carbon dioxide

CSA Cross-sectional area

CVD Cardiovascular disease

CYP450 Cytochrome P450

D Diameter

DBP Diastolic blood pressure

DICOM Digital imaging and communications in medicine

ECG Electrocardiogram

EDHF Endothelial dependent hyperpolarizing factors

eNOS Endothelial nitric oxide synthase

FMD Flow-mediated dilation

GI Glossopharyngeal insufflation

HR Heart rate

IBM Involuntary breathing movement

L Lower

MAP Mean arterial blood pressure

NO Nitric oxide

NO_2^- Bioactive nitrate

NO_3^- Inorganic nitrate

O_2 Oxygen

O_2^- Superoxide anion

$ONOO^-$ Peroxynitrite

PG Prostoglandins

PR Personal record

PRH Peak reactive hyperemia

RBC Red blood cells

RV Residual volume

SBP Systolic blood pressure

SCUBA Self-contained underwater apparatus

SNA Sympathetic nerve activity

TLC Total lung capacity

U Upper

U/S Ultrasound

V Velocity

VC Vital capacity

VO_2 Oxygen volume

1. Literature Review

1.1 Professional breath-hold divers

Interest in breath-hold diving (BHD) first took place over 2000 years ago, in the ancient Greek mythological traditions of the Ama. These women dove to catch fish off the coast of Japan and Korea for 30-90 s bouts, up to 4 hours a day with approximately 25-30% of their time underwater (Hurford et al., 1990). The physiological adaptations involved in long duration breath-holds has attracted the attention of physiologists over time (Hong, 1988; Kang, Song, Suh, & Hong, 1963). This interest in breath-holding has turned into an international and professional-scale sport. The Association Internationale pour le Développement de l'Apnée (AIDA) and the World Confederation of Underwater Activities (CMAS) are the two primary governing bodies that set the standards for safety, comparability of World Records as well as free dive education. AIDA and CMAS categorized different streams of BHD, based on depth, distance and maximum breath-hold associated with a single breath. Occupationally, BHD sports include spearfishing, underwater photography, underwater rugby, hockey, target shooting as well as synchronized swimming. Professional BHD is categorized into dynamic (exercising) and static (non-exercising) scenarios, with each characterized by holding one's breath (also called apnea) as long as physically possible. When divers swim underwater the furthest distance possible in a singular breath, it is characterized as dynamic. When athletes hold their breath face down in the water, it is characterized as static apnea. In all types of breath-hold diving, these athletes are putting themselves in situations of severe hypoxia and hypercapnia. Moreover, the training for these competitions involve repeated bouts of severe hypoxia and hypercapnia each day.

1.2 Hypoxic scenarios

Hypoxia is defined as the deficiency of oxygen supply to an organ, such as the brain. The brain has a high metabolic demand, and although it represents only 2% of total body weight, it accounts for approximately 20% of resting total body O_2 consumption. On average the rate of O_2 consumption of the entire brain (weighing 1,400g) is 49 mL

O_2/min (Clarke & Sokoloff, 1999). Nevertheless, the brain is not capable of storing oxygen, thus needs constant circulatory regulation to maintain cerebral oxygen delivery (Hoiland, Bain, Rieger, Bailey, & Ainslie, 2016). Cessation of oxygen supply to the brain results in unconsciousness within 4-6 seconds (Smith, Clayton, & Robertson, 2011), and brain death within minutes (Hoiland et al., 2016). Situations of hypoxia, as seen in professional BHD, present a critical challenge to brain oxygen supply. Many cardiovascular, cerebrovascular and metabolic mechanisms play a role in conserving oxygen in divers during hypoxia. The maintenance of oxygen delivery during hypoxia is possibly due to prioritization of oxygen-rich blood flow to the brain, as well as efficiently and sparingly using the available oxygen (Bain, Ainslie, Drvis, Dujic, & Macleod, 2018). With those mechanisms involved, the current world record for competitive static breath hold is 11 min 35 s by frenchman Stéphane Mifsud (www.aidainternational.org).

1.3 Mechanisms

The ability for elite apneists to breath-hold beyond normal physiological ranges depends on two fundamental principles: 1) oxygen storage capacity (i.e. increased lung capacity), and 2) oxygen conservation. The latter is achieved through cardiovascular and metabolic adjustments, termed the Diving Reflex (Gooden, 1994). Specifically, dive responses are associated with vagally-mediated bradycardia (decrease in heart rate) and sympathetically-mediated splenic and peripheral vasoconstriction (Foster & Sheel, 2005; Schagatay, 2009). Similarly, Weddell seals have exceptional diving physiology, as they are capable of remaining submerged for over an hour and reach depths of over 500 m (Kooyman, 1981). This seemingly simple process of oxygen conservation in mammals involves a number of complex physiological mechanisms. In order to understand possible impairments with BHD, it is crucial to understand the ways in which they physiologically adapt to conserve their oxygen.

1.3.1 Vagally-mediated bradycardia

A static breath-hold causes heart rate (HR) to decrease substantially by ~30-50 beats per minute (Perini et al., 2008). The benefit of apnea-associated bradycardia is the

subsequent reduction in myocardial oxygen consumption, and slower rate of total oxygen desaturation. For elite apneists, this means an overall increased maximal breath-hold duration. For example, the administration of a cardiac-specific blockade *esmolol* to minimize neutrally-medicated heart rate and contractility and thereby pharmacologically reduce oxygen consumption by the heart, accentuated the reductions in HR, while increasing the maximal static apnea duration by 33s compared to placebo. Surprisingly, oxygen saturation levels at the end of the breath-holds were found similar between placebo and *esmolol* conditions. These data suggest that increased apnea duration with cardiac blockade was associated with reduced oxygen consumption by the heart (Hoiland et al., 2017). Unsurprisingly, the longest apnea durations correlated (R=0.65) to greater bradycardic responses (Schagatay, 2009, 2010).

1.3.2 Peripheral vasoconstriction - skeletal muscles

During an apnea, peripheral vasoconstriction primarily occurs in skin and skeletal muscle tissues (Ferretti, 2001), but can also occur at the heart, kidney and spleen (Bakovic et al., 2003; Kyhl et al., 2016; Mijacika et al., 2017). Evidence for peripheral vasoconstriction with apnea was originally found in diving mammals by Scholander (1940) where lacerated skeletal muscles in seals bled only when the seal was breathing, but halted during underwater facial submersion (Blix & Folkow, 2011). In humans, arterial blood flow in the limbs (via venous occlusion plethysmography) decreased during 30 s of static breath hold from ~21.0 to 13.5mL/min in the finger (100 mL of tissue), along with an increase in arterial pressure. The rise in blood pressure indicates that the reduction in peripheral blood flow occurs due to a reduction in conductance, rather than a decrease in perfusion pressure (Heistad, Abboud, & Eckstein, 1968). The same study concluded that peripheral vasoconstriction occurs due to increased sympathetic activity, as the vasoconstrictor response did not change in the presence of parasympathetic blockade *atropine* (despite preventing vagally-mediated bradycardia). Current research corroborates that increased sympathetic activity is associated with increased peripheral vasoconstriction, and thus decreased peripheral blood flow. The original involvement of the sympathetic nervous system stemmed from the study who examined a ~2000% increase in muscle sympathetic nerve activity from baseline during maximal dry static

breath-hold (Heusser et al., 2009; Steinback, Salmanpour, Breskovic, Dujic, & Shoemaker, 2010). These early sympathetic responses occur at the end inspiratory apnea due to the concurrent fall in blood pressure. Lastly, the extent of peripheral vasoconstriction and blood flow trends, are easily influenced by breath-holding conditions (i.e. duration, competition level, years of training (Bain et al., 2017).

1.3.3 Peripheral vasoconstriction - splenic contractions

The spleen is a dynamic blood cell reservoir, containing 8% of total red blood cells in humans, densely packed and measuring ~200-250 mL (Koga, 1979). During breath-hold, splenic contractions cause circulating hemoglobin and hematocrit levels to increase (Bakovic et al., 2005). This response will enhance oxygen transport throughout the body. The duration of the breath-hold, and whether it is performed underwater, influences the magnitude of the splenic contraction (Espersen et al., 2002). At the end of a five minute static breath-hold, splenic contractions caused hematocrit and hemoglobin levels to increase by 4% (Bain et al., 2016, 2017). The rapid onset of the contractions (Palada et al., 2008) insinuates that the initial stimulation is sympathoexcitatory in nature, and that these contractions are maintained by catecholamines circulating in the blood (Bakovic et al., 2005).

In summary, the primary role of peripheral vasoconstriction during breath-hold is to prioritize oxygen-rich blood flow to the brain, while simultaneously attenuating the decline in arterial oxygen saturation by contracting the spleen to increase circulating hemoglobin concentrations through the body (Espersen et al., 2002), and by shifting the hypoxic skeletal muscle to a non-oxidative type of metabolism (Ferretti, 2001). The latter has been found more rigorously in dynamic BHD as a leading role for oxygen conservation (Andersson & Evaggelidis, 2009; Andersson, Line, Fredsted, Schagatay, & Fredsted, 2004; Marongiu, Crisafulli, Ghiani, & Roberto, 2014), yet little evidence proves its role in static BHD.

1.4 Training and Physiological Adaptations

Professional apneists typically have a total BHD training load of 6.2 ± 0.6 hours/week, with an additional 6.3 ± 0.7 hours/week on other physical training, one month prior to competition. Breath-hold training includes serial and maximal static dry and immersed apneas, as well as walking apnea and dynamic apnea in a pool. Physical training includes endurance capacity such as running, cycling or biking (Schagatay, Richardson, & Lodin-Sundstrom, 2012). Aside from physical training, many modifiable physiological processes contribute to BHD capabilities.

The ability for apneists to successfully hold their breath for longer than 5 minutes is attributed to a combination of genetic (Eftedal, Flatberg, Drvis, & Dujic, 2016) as well as modifiable physiological and psychophysical factors (Fig. 1). Although a large number of mechanisms and processes are responsible for prolonged static-breath hold durations; apnea preparation (Engan, Jones, Ehrenberg, & Schagatay, 2012; Ghiani et al., 2016) maximal oxygen storage ability (VO_2 storage)(Bakovic et al., 2005, 2003; Schagatay, Andersson, & Andersson, 2001), minimal oxygen consumption (VO_2 economy)(Hoiland et al., 2017) and minimal carbon dioxide (CO_2) buildup (Heusser et al., 2009) are primary determinants of successful BHD. However, within each mechanism exists a number of genetic, physiological or psychological factors that allow that modification. Chronic strategies include preparatory apneas, increased dietary nitrate (Engan et al., 2012), fasting (Lindholm, Conniff, Gennser, Pendergast, & Lundgren, 2007) and decreased muscle tonus through relaxation, where acute strategies include decreased mental excitation and increased ventilation. Specifically in regards to nutrition, a study found that maximal apneic duration was elongated by 11% after supplementing 2.5 hours prior to BHD with NO_3^- rich (~5.0 mmol of NO_3^-) beetroot juice, compared to a placebo (Engan et al., 2012). It is known that inorganic NO_3^- is rapidly metabolized in the gut to bioactive nitrate (NO_2^-) and eventually create NO (Lundberg & Govoni, 2004), and that acute and chronic terms of NO_3^- supplementation can therefore decrease metabolic cost and increase BHD duration (Engan, Jones, Ehrenberg, & Schagatay, 2012). VO_2 storage is modified by the following factors: increased glossopharyngeal insufflation (Overgaard, Friis, Pedersen, & Lykkeboe, 2006), increased vital capacity, decreased residual volume,

increased inspiration (Espersen et al., 2002) etc. Lastly, VO_2 economy can be influenced by factors such as attenuated HR (Hoiland et al., 2017) through increases in both parasympathetic activity (Lemaitre, Chowdhury, & Schaller, 2013) and arterial compliance (Tanaka, Tomoto, Kosaki, & Sugawara, 2016), as well as increased peripheral vasoconstriction due to increased sympathetic nerve activity etc. (Dujic et al., 2008). Modifiable techniques such as improved relaxation, increased motivation and stress tolerance also contribute to increased breath-hold durations (Ostrowski, Stanula, Pilch, & Maszczyk, 2012). Additionally, residents of the Himalayan valleys who experience O_2 levels approximately 40% lower than those at sea level (Beall, 2007) develop a favorable phenotype that involves hypoxia-inducible factor pathway genes (Lorenzo et al., 2014), transcription factors that induce the transcription of hundreds of genes involved in maintaining oxygen levels in tissues (Semenza, 2007). Although no research has examined their ability to maintain breath-hold dives, this chronic adaptation to hypoxia is a possibility for occurrence in the BHD community as well.

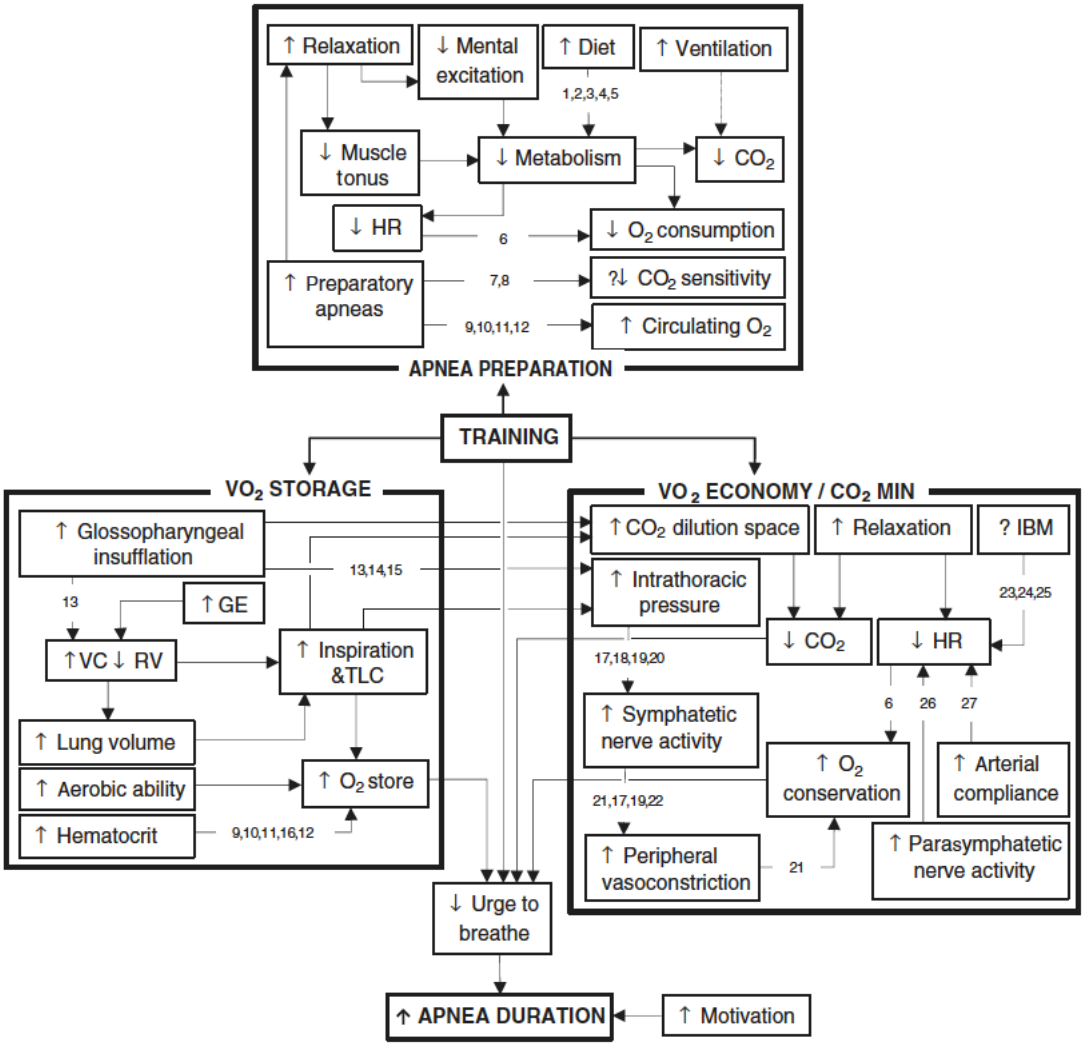

Figure 1. Adaptation with breath-hold diving. HR, heart rate; VC, vital capacity; RV, residual volume; IBM, involuntary breathing movement; TLC, total lung capacity; GE, glossopharyngeal insufflation. Permission from Bain et al., 2018.

1.5 Link to problems

Breath-hold diving has been linked to a series of acute dangers, such as glossopharyngeal insufflation (GE)(Chung et al., 2010; Mijacika & Dujic, 2016) barotrauma, drowning, syncope from diminished cardiac output with GI (Lindholm & Lundgren, 2009), and cardiac arrest during breath-hold (Hong, Song, Kim, & Suh, 1967). Long-term impacts contrarily, remain vastly understudied and understood. Interestingly, divers performing >150 dives a day (e.g. spear fishing or diving practice) will mimic some of the

physiological changes associated with the obstructive sleep disorder, termed sleep apnea. Nonetheless, whereas cerebrovascular CO_2 reactivity is reduced in sleep-breathing conditions, (Prilipko, Huynh, Thomason, Kushida, & Guilleminault, 2014) it remains unchanged in BHD, (Ivancev et al., 2007) as are sympathetic baroreflex gain and respiratory muscle SNA modulation that are found in those with sleep apnea (Steinback, Salmanpour, Breskovic, Dujic, & Shoemaker, 2010) but not BHD. Further, competitive apneists do not experience autonomic dysregulation commonly found in sleep apnea (Dempsey, Veasey, Morgan, & Donnell, 2010). Conversely, competitive BHD does yield long-term neurological problems such as slower Stroop test responses and more errors on interference card tests. Maximal breath-hold abilities and years of training were positively and strongly correlated (r=0.73 and r=0.79, respectively) with the cognitive neurological findings, suggesting that BHD training also causes persistent short-term memory impairments (Billaut, Gueit, Faure, Costalat, & Lemaitre, 2018). Additionally, there is evidence of impaired flow-mediated-dilation and decreased circulation NO levels from repetitive hypoxia during transient breathing cessation in individuals with obstructive sleep apnea (Kato et al., 2000; Noda, Nakata, Koike, & Miyata, 2007). The vascular endothelial layer is fundamental to health and normal vascular control. Yet, the impact of BHD training has not been examined.

1.6 Biology/Physiology

Endothelial cells line the arterial vasculature and this monolayer contributes to, and/or regulates vascular responses to both mechanical and chemical environment changes. The endothelium has many pathological and physiological roles including control of thrombosis, inhibition of leukocyte and platelet cell adhesion, and promotion of intra-arterial permeability (Celermajer, 1997; Rubanyi, 1993; Vane, Anggard, & Botting, 1990). Additionally, the endothelium releases many vasoactive compounds including prostacyclins, endothelins, endothelial cell growth factors, interleukins, and nitric oxide (Furchgott & Zawadzki, 1998). These compounds are responsible for regulating diameter, tone and structure of the vasculature, thus balancing oxygen supply with the neighboring tissues' metabolic demands. Nitric oxide (NO) is the major contributor to vasodilation of vessels (Singel & Stamler, 2005) and has been examined

for decades. Shear stress-sensitive ion channels exist in these endothelial cells (Cooke, Rossitch, Andon, Loscalzo, & Dzau, 1991; Lansman, Hallam, & Rink, 1987; Olesen, Clapham, & Davies, 1988) and in response to increases in blood flow and the ensuing shear, they release NO (Moncada, Radomski, & Palmer, 1988). This release causes vasorelaxation of the smooth muscle, and subsequently diameter change. These vasodilatory responses can be measured through flow-mediated dilation.

1.7 Methods – flow-mediated dilation

Endothelial health and function are often measured by a technique called flow-mediated dilation. Flow-mediated-dilation (FMD) was first proposed in humans as a reactive hyperemia endothelial function test by Celermajer and colleagues (Celermajer et al., 1992). It is defined as downstream vasodilation of conduit arteries in response to internal shear stresses of the vessel walls, following a period of distal limb ischemia via a pressure cuff. These changes in artery dilation are often measured via Doppler ultrasound. Upon cuff release, the inflow of blood into the artery creates a moment of reactive hyperemia, elevating the shear stimulus for several minutes, causing the endothelial-mediated FMD response. From this technique, data on artery diameter, blood flow velocity, total blood flow and shear stress are available.

This FMD technique adequately stimulates endothelium-mediated dilation as it provides a dose-dependent measure of endothelial function over various durations of ischemia in different arteries (i.e. femoral, brachial). In these models, the use of NO antagonists attenuated the FMD response, i.e. NO synthase blockade N-monomethyl-L-arginine (Cooke et al., 1990; Hutcheson & Griffith, 1991) supporting the important role of NO in this response. Although some contradicting research claims that NO combined with prostaglandins (PG) do not play an essential role in rapid vasodilation at the onset of exercise (Saunders, Dinenno, Pyke, Rogers, & Tschakovsky, 2005), there is definitely an endothelial signal in which NO and PG mediate its effect.

Figure 2 represents the steps of FMD as they begin with the generation of a shear-stress stimulus (*step 1)*, and result in vessel diameter change (*step 6)*. Blood flow shear stress on the cell membrane is sensed/detected by mechanosensitive structures. These structures include the glycocalyx, primary cilia, and mechanosensitive ion channels (Davies, 2009). This mechanotransduction activates a signaling cascade that produces vasodilator substances (such as NO) (*step 2)*. The vasodilators involved in FMD depend on the nature of the shear stress stimulus (Pyke & Tschakovsky, 2005) and the endothelial phenotype – whether with or without EDHF compensatory capacity (Pyke et al., 2010). Next, the vasodilators diffuse from the endothelial cell into the smooth muscle cell (*step 3)*. Nitric oxide may react with reactive oxygen species (ROS), thus causing a decrease in its bioavailability (Rush, Denniss, & Graham, 2005). The FMD-specific vasodilators trigger a signaling cascade that lowers calcium concentration and causes vasorelaxation in the vascular smooth muscle cell (*step 4)*. The diameter of the vessel will dilate for the given degree of smooth muscle relaxation *(step 5)* (Lind, 2007).

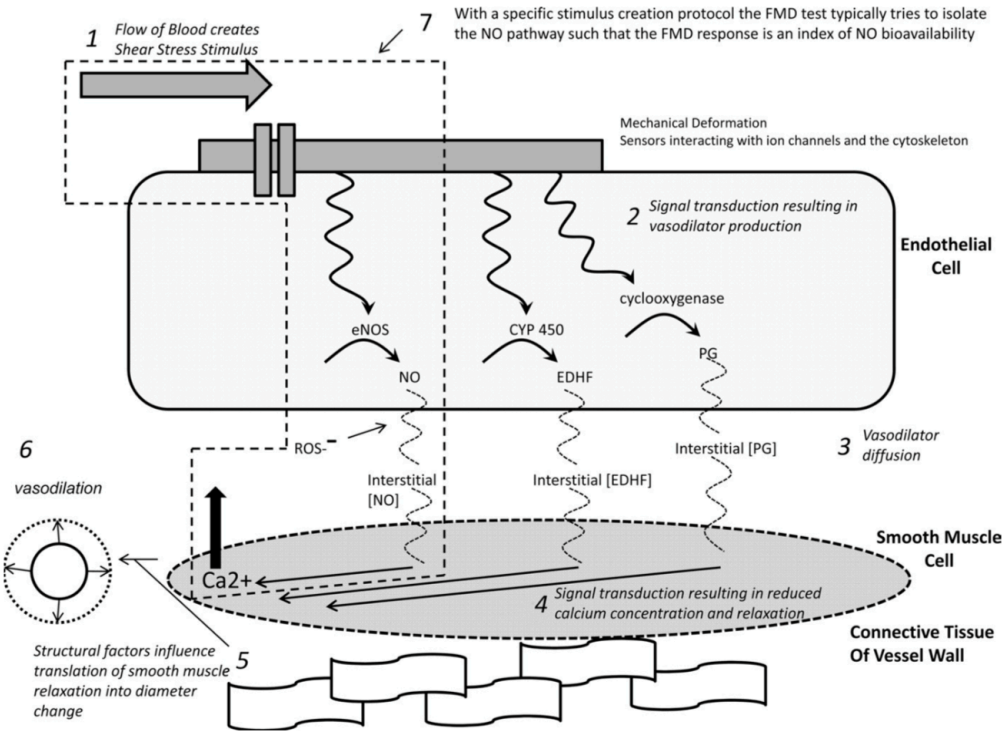

Figure 2. Schematic representation of steps involved in flow-mediated dilation (FMD). eNOS, endothelial NO synthase; NO, nitric oxide; CYP 450, cytochrome

P450; EDHF, endothelial-derived hyperpolarizing factor; PG, vasodilatory prostaglandins. (Permission from Thijssen et al., 2010).

This reinforces the original works of Celermajer and Deanfield (1992), that FMD technique is an endothelial dependent and NO-specific index of endothelial function. The ultrasonic assessment of FMD in response to occlusion-induced hyperemia is now established as a reliable, non-invasive measurement of endothelial function (Uehata et al., 1997).

1.7.1 Sensitivity to age and health

FMD has become increasingly popular in clinical studies as a strong predictor of cardiovascular events in CVD patients (Karatzis et al., 2006; Modena, Bonetti, Coppi, Bursi, & Rossi, 2002). Furthermore, FMD is able to provide independent prognostic information that exceeds traditional risk factors (Fathi, Haluska, Isbel, Short, & Marwick, 2004; Meyer et al., 2005; Neunteufl et al., 2000; Patti et al., 2005). Even in asymptomatic patients, there is a modest association of the prognostic role of FMD (Fathi et al., 2004; Frick et al., 2006; Yeboah, Crouse, Hsu, Burke, & Herrington, 2007). Although strong associations are found with medical conditions, FMD sensitivity decreases with age with individuals whom arterial distensibility may be limited (Witte et al., 2005; Yeboah et al., 2009). A recent meta-analysis supported the proposition that FMD is at least as predictive as traditional factors in predicting future cardiovascular events (Inaba, Chen, & Bergmann, 2010) and may provide important prognostic information for humans.

1.7.2 FMD as a diagnostic tool

Flow-mediated-dilation studies larger peripheral conduit vessels, yet is representative of more clinically relevant coronary circulation (Takase et al., 1998). Due to the close nature of the relationship between peripheral and coronary circulation, FMD assesses endothelial dysfunction in clinical and asymptomatic patients. Also, FMD has increasingly been helpful in numerous studies to examine the mechanism underlying acute or chronic stimuli that alter vascular function and risk (e.g. smoking, hypertension, exercise training)(Ghiadoni, 2001; Karatzis et al., 2006). The reduction of NO

availability, or reduced NO bioavailability has been associated with the term “endothelial dysfunction” which is a common predictor of many cardiovascular diseases (Cai & Harrison, 2000).

1.8 Methodological Issues

Small alterations in the methodological approach of FMD impacts the magnitude of the FMD response (Black, Cable, Thijssen, & Green, 2008; Doshi et al., 2001; Mullen et al., 2001). Although there are standardized guidelines to the technique, studies have identified important physiological and technical issues that could influence the validity, reproducibility and interpretation of FMD studies. These include technical issues pertaining to duplex ultrasound assessment of FMD, and methodological considerations pertaining to FMD assessment.

1.8.1 Ultrasound

The ultrasound technique for measuring FMD is relatively simple and non-invasive. However, appropriate training, equipment and skill are required for accurate and reliable measurements. The measurement accuracy depends on identifying a clearly defined arterial wall (Fig. 3). While imaging a blood vessel in the longitudinal plane, the double lines of Pignoli – (intima-media thickness)(Pignoli, Tremoli, Poli, Oreste, & Paoletti, 1986) should be visible, as distinguishable boundaries allowing for precise diameter measurement.

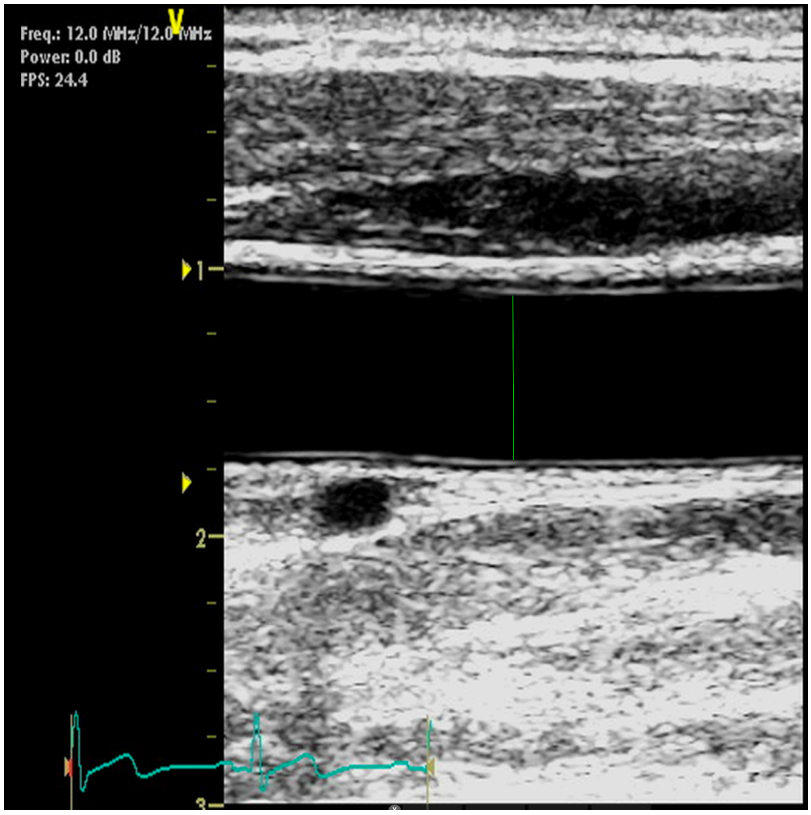

Figure 3. Representation of the vascular boundaries on a brachial artery. The green line indicates the measurement tool for collecting diameter.

The ultrasound probe is classified per frequency range in megahertz (MHz), which is inversely proportional to the optimal depth for imaging. For superficial vessels, such as the brachial artery, a 7-14 MHz high resolution probe is optimal. Lower frequencies have the capability of measuring changes in less superficial vessels. Duplex mode (B-mode) allows simultaneous collection of vessel diameter and flow velocity (Fig. 4).

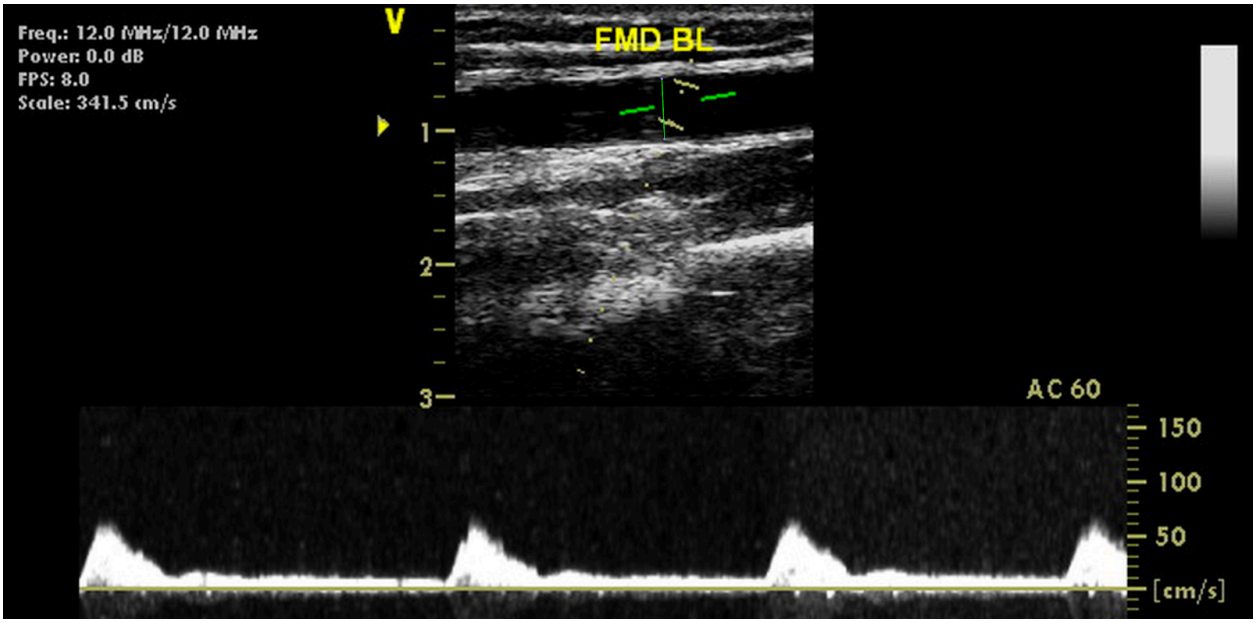

Figure 4. Representation of Duplex Mode on Vivid I ultrasound, with collection of vessel diameter (top) and blood flow velocity (bottom) taken at baseline, over 20 seconds. Scale is blood flow velocity in cm/s. AC 60 is the insonation angle.

These collective measurements allow the calculation of shear rate and blood flow (as well as shear rate area under the curve, AUC). AUC is a sum of all the velocity points under the curve from the moment of cuff deflation until the end of the FMD protocol (Fig. 6). This value is important, as it allows us to assess the extent of the shear stress stimulus an individual will receive.

The advantage of Duplex mode is that it demonstrates the cumulative effect of shear stress on the artery, which is proposed to be the major stimulus for the FMD response (Mitchell et al., 2004). Although M-mode ultrasound is useful for measuring diameters during a series of cardiac cycles (e.g. during systole vs. diastole), it is not possible to simultaneously measure blood flow velocity. The angle steer and the insonation angle correction are other considerations for accurate measurements. Duplex ultrasound incorporates a Doppler beam aperture that can be steered 20-30° of the center of the B-mode imaging beam, allowing Doppler shifts to be achievable at an angle of 60° between the beam and the vessel orientation.

Lastly, analysis of velocity signal can be calculated using either the peak (peak Doppler shifts) or mean velocity (intensity weighted mean of all Doppler shifts). The peak-velocity approach measures only the fastest moving blood cells, located at the middle of the vessel, whereas mean velocity takes the average of all adjacent blood flow velocity profiles within the cross-section of the vessel (Fig. 5).

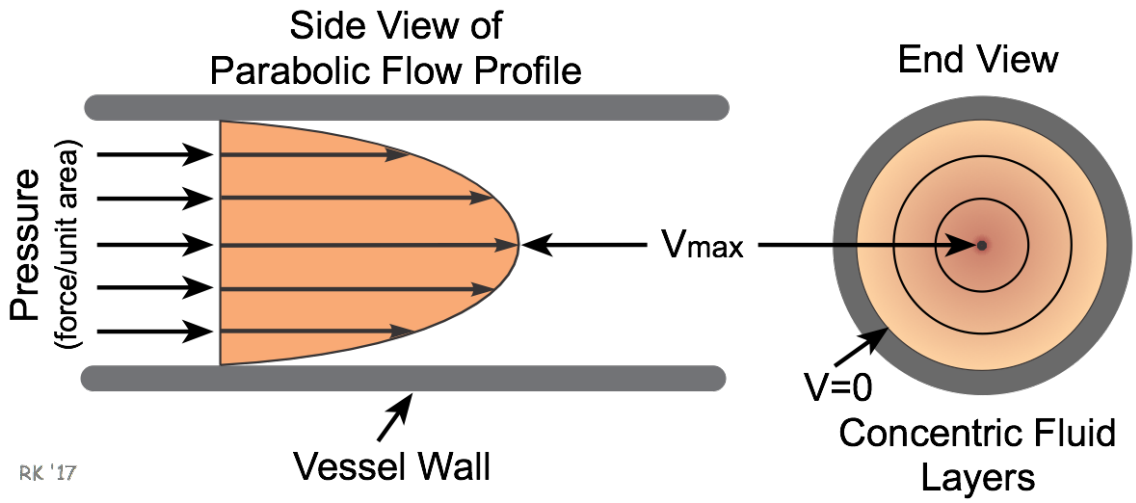

Figure 5. Schematic representation of parabolic flow profile, where peak velocity would only capture the Vmax, and mean velocity would capture all adjacent velocity profiles. Requested permission from www.cvphysiology.com.

It is correct to assume that half of the peak velocity is representative of mean velocity in laminar flow (Li, Hoskins, Anderson, & McDicken, 1993). Thus, to capture the mean flow velocity from the Doppler spectrum, the mean-weighted approach measures the blood velocity from the entire cross-section of the blood vessel, including both the fast red blood cells (RBCs) moving in the center but also the slower moving cells close to the lamina of the vessel. This latter approach can be limited, as the narrow ultrasound beam does not always capture the slower moving cells at the laminae (Thrush & Hartshorne, 2004) resulting in a velocity overestimation of approximately 33% (Evans & McDicken, 2000). Both peak and mean velocity measurements offer advantages and disadvantages, and should not be used interchangeably (Evans, Schlindwein, & Levene, 1989), nor in the same study.

1.8.2 Technical Issues with Ultrasound: diameter and velocity assessment

High-resolution B-mode ultrasound is the standard tool of choice for measuring artery diameter in FMD protocols. The main challenge with B-mode imaging is clearly defining vascular boundaries. When available, and due to the recent understanding of the importance of quantifying shear stress during the FMD protocol, (Padilla et al., 2008; Pyke & Tschakovsky, 2005; Pyke & Tschakovsky, 2007) a Duplex ultrasound is recommended, due to its simultaneous acquisition of B-mode diameter and pulsed-waved Doppler velocity signals. This concurrent detection of both signals is detected by the same transducer, which have competing requirements for optimal data acquisition. B-mode images are of greater quality when the ultrasound probe is perpendicular to the vessel orientation (90°), whereas the optimal Doppler signals require parallel incidence with the direction of blood flow (0°) (Kremkau, 2002; Oates, 2001; Thrush, 2004); however, the ideal insonation angle is 60°. As the angle increases over 60°, the error associated with incorrect angle increases exponentially (Kremkau, 2002; Oates, 2001; Thrush & Hartshorne, 2004). Consequently, the recommended Doppler beam-vessel insonation angle for clinical ultrasound in order to yield adequate results (Hoskins, Thrush, Martin, & Whittingham, 2002; Kremkau, 2002) with reasonable levels of measurement error (Polak, 2004) is ≤60° in relation to blood flow. Therefore, a compromise must be reached to uphold fundamental principles of both modalities, as well as provide the best signal and images without loss of quality (Oates, 2001).

1.9 Calculation of FMD

FMD or endothelial function is thus measured by comparing the increase in arterial diameter as a consequence of the reactive hyperemia to the baseline diameter, expressed as percent change of diameter (%FMD). This is calculated as:

$$FMD = \frac{(peak\ diameter - baseline\ diameter)}{baseline\ diameter} \times 100\%$$

Vessel type and size must be considered, as they can influence the contribution of NO (Shimokawa et al., 1996), and there is still debate that vasodilation mediated by endothelium is primarily the result of NO (Pyke et al., 2010; Tschakovsky & Pyke, 2005). A normal physiological range for healthy %FMD response is between 2-13%, where a greater number represents a greater vasodilatory response to shear stress (Clarkson et al., 1999; Neunteufl et al., 1997).

1.10 Calculation of peak reactive hyperemia

Reactive hyperemia is defined as the transient period of blood velocity increase as a result of a period of ischemia. Peak reactive hyperemia (PRH) is the maximal velocity value obtained post cuff-occlusion. PRH occurs within ~4-7 s post-cuff release, returning to baseline velocity levels within approximately two minutes (Corretti et al., 2002). Reactive hyperemia is thought to be due to the interplay of myogenic and metabolic factors (Sparks & Belloni, 1978). Specifically, prostaglandins (Carlsson, Sollevi, & Wennmalm, 1987; Herbaczynska-Cedro, Staszewska-Barczak, & Janczewska, 1974; Kilbom & Wennmalm, 1976) adenosine (Bockman, Berne, & Adenosine, 1976; Carlsson et al., 1987; Dobson, Rubio, & Berne, 1971), and ATP-sensitive potassium channels (Aversano, Ouyang, & Silverman, 1991; Kanatsuka et al., 1992) are metabolic factors contributing to reactive hyperemia. Additionally, reactive hyperemia in the human forearm includes a myogenic phenomenon through external compression of the cuff on the downstream vasculature. Although there is a metabolic component involved in reactive hyperemia, it primarily reinforces the myogenic response (dominant in the first 30s), but is unable to relax the vascular smooth muscle when tension is maintained phasically (Carlsson, Sollevi, & Wennmalm, 1987).

1.11 Calculation of shear rate

The hemodynamics inside blood vessels cause two types of superficial stresses near the vessel walls: 1) circumferential stress, and 2) shear stress. For the purpose of this thesis, we will focus primarily on the latter. The direction of the blood flow velocity vector against the vessel wall dictates the direction of the shear stress. Unlike normal stresses

due to blood pressure that are transferred to all layers of the vessel wall (intima, media and adventitia), shear stress is primarily applied to the inner layer of the arterial wall, the vascular endothelium. The direct (normal) as well as the indirect (shear) stresses applied work collectively to regulate blood vessel diameter depending on both vascular wall elasticity (distensibility) and endothelial function (endothelial induced vasodilation). Shear stress is directly related to the viscosity and the velocity of the blood, and inversely related to the diameter of the vessel. In conduit arteries where flow is laminar and unidirectional, shear stress can be calculated as follows:

$$Shear\ stress = viscosity \times \frac{velocity}{diameter}$$

Therefore, shear rate is defined as the "rate at which adjacent layers of fluid move with respect to each other". The magnitude of the shear rate (expressed in reciprocal seconds) is representative of the shape of the velocity profile for that given stimulus. Given that blood is a Newtonian fluid with constant viscosity, the flow is steady and the vessel is cylindrical, thus Poiseuille's law can be applied to determine shear rate as follows:

$$Shear\ rate = \frac{8 \times u}{d}$$

where *u* is the average velocity, and *d* is the vessel diameter.

Total shear rate (cm/s x s) is calculated by measuring the area under the velocity curve (AUC), and must be presented in results. This area includes the moment of cuff deflation until the end of the FMD stimulus (Fig. 6-7).

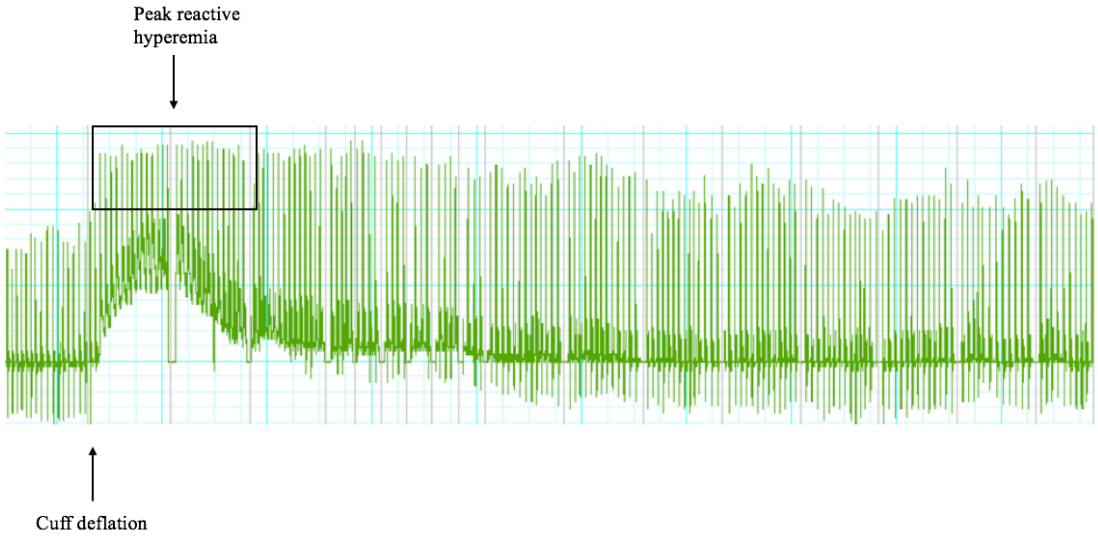

Figure 6. Schematic representation of blood flow velocity profile at cuff release, until the end of the protocol. Area under the curve is collected as outcome of total shear (NU).

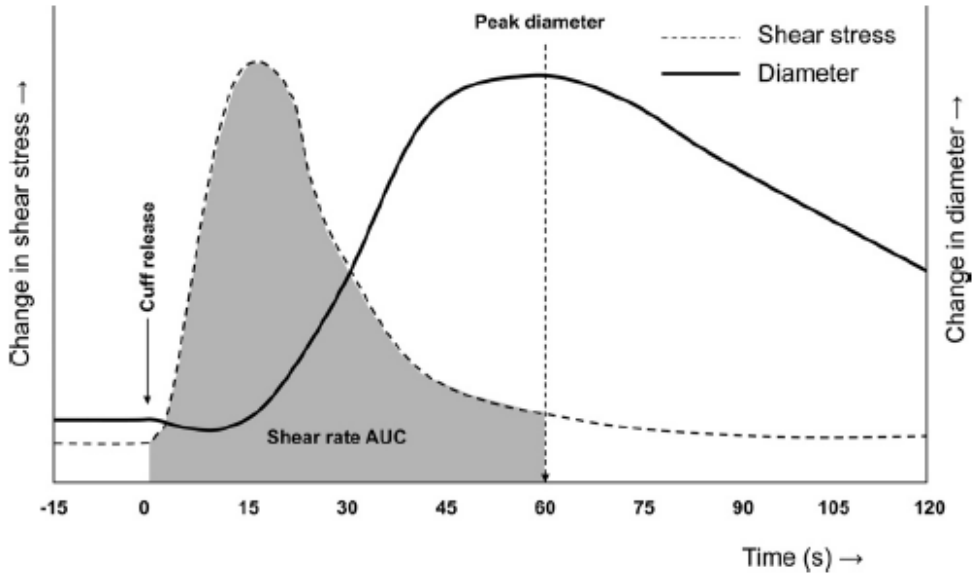

Figure 7. Schematic representation of diameter and shear-stress response following cuff deflation. Gray area represents the area under the curve (AUC). Permission from Thijssen et al., 2010.

The objective in FMD studies is to achieve relatively consistent shear stimulus to vascular endothelium across all participant groups, even with potential changes in artery

diameter and flow. Recent studies have proved that the addition of blood viscosity into the calculation of shear stress does not significantly impact the values nor the interpretation of FMD results (Padilla et al., 2008). To examine differences between participants on shear, the following equation calculates normalized shear rate of individual %FMD responses:

$$Normalized\ \%\ FMD = \frac{individual\ \%\ FMD}{total\ individual\ shear}$$

The endothelium responds to such shear stresses through various physiological mechanisms, depending on the magnitude of the stimulus.

1.12 FMD recommendations

Certain methodological and technical guidelines exist for FMD assessment of conduit arteries in humans. In regards to subject preparation, they must be in a supine posture to control for orthostatic changes. The cuff must be placed distally from the imaged artery, as cuff placement will influence the nature of FMD response. Specifically, dilators such as NO as well as myogenic responses may be affected when the cuff is placed above the imaged artery (Doshi et al., 2001). In that, the increase in arterial pressure on release of the cuff in the proximal position could evoke a myogenic response that would constrict the brachial artery, as opposed to dilate it. Concurrently, a myogenic dilation would likely occur prior to cuff deflation due to reductions in arterial pressure distal to the cuff during the ischemic period, as pressures equilibrate across the arterial-venous circulations. Thus, different mechanisms likely are involved for proximal vs. distal placement of the occlusion cuff. The distal cuff placement more specifically focuses on shear-related mechanisms whereas the proximal cuff placement would also engage myogenic contributions (Betik, Luckham, & Hughson, 2004)(Fig. 8).

Figure 8. Schematic drawing of ultrasound imaging of the brachial artery with lower cuff placement and transducer position above the antecubital fossa. Modified with permission from Corretti et al., 2002.

Further, the cuff must be inflated for a minimum of five minutes for the most easily-tolerated yet effective occlusion. An occlusion greater than five minutes, has been found to prolong the duration of reactive hyperemia as well as evoke a non-NO-mediated response (Mullen et al., 2001). A careful health history should be taken regarding medications and health conditions, as some drugs have effects on endothelial and vascular function. FMD can be influenced by dietary intake (Berry et al., 2000); therefore, it is recommended that participants avoid exercise or food/drinks containing caffeine and alcohol for ≥8 hours (Hijmering et al., 2002; Parker, Ridout, & Proctor, 2006). The time of day may influence FMD results (Jones, Green, George, & Atkinson, 2009; Otto et al., 2004; ter Avest, Holewijn, Stalenhoef, & de Graaf, 2005); thus, tests should be conducted at a similar time of day for between-group studies. Additionally, testing should be conducted in a quiet, temperature controlled-room, as the sympathetic nervous system activation can alter FMD results (Dyson, Shoemaker, & Hughson, 2005; Hijmering et al., 2002; Lind, Johansson, & Hall, 2002; ter Avest, Holewijn, Stalenhoef, & de Graaf, 2005). Due to differences in hormone concentration in premenopausal women, female participants should be tested in a standardized phase of their menstrual cycle - preferably day 1-7 in the follicular phase where the concentration of hormones are the lowest (Hashimoto et al., 1995; Williams et al., 2001). During FMD protocol, baseline diameter must be examined for one minute prior to cuff inflation, as established

by Celermajer and Deanfield (1992) in the first FMD study, as it continues to be a valid measurement of the "true" diameter. Measurements of absolute baseline diameter, as well as post-deflation diameter are required, the latter lasting ≥3 minutes. This 180 s period is based on the assumption that most peak measurements in brachial artery diameter would occur in the first 120 s after cuff release (Black, Cable, Thijssen, & Green, 2008). This timeline correlates with the moment of maximal vessel diameter occurring at 45 to 60 s after peak reactive hyperemia (Corretti, Plotnick, & Vogel, 1995; Uehata et al., 1997), and 90 s after the moment of cuff deflation (Fig. 9).

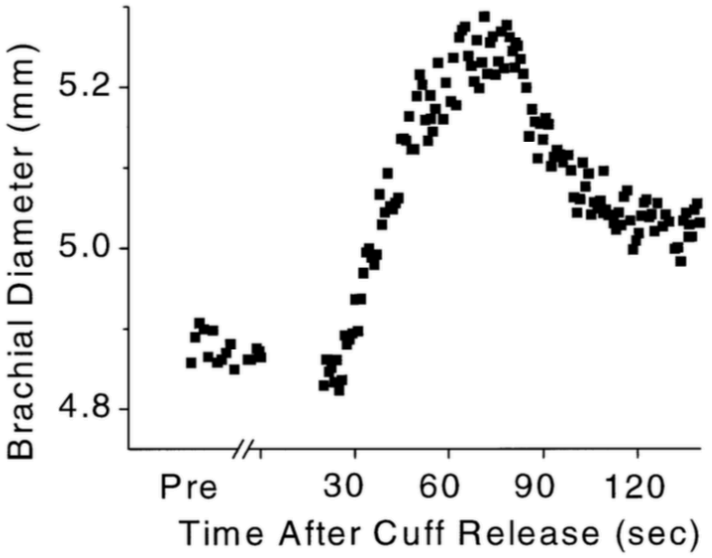

Figure 9. Time course of brachial artery flow-mediated vasodilation (FMD) in a healthy individual. Note that maximal brachial diameter occurs at approximately 45 to 60s after peak reactive hyperemia. Permission from Corretti et al., 2002.

Measurements of the brachial diameter should be taken at end diastole to decrease any influence of vascular compliance on diameter measurements (Corretti et al., 2002). Systole and diastole in relation to diameter size can be seen and measured in M-mode on the ultrasound machine strictly for baseline images (Fig. 10). The remainder of the FMD protocol should be performed using B-mode, to ensure the complete velocity time course

during each image collected (Fig. 4).

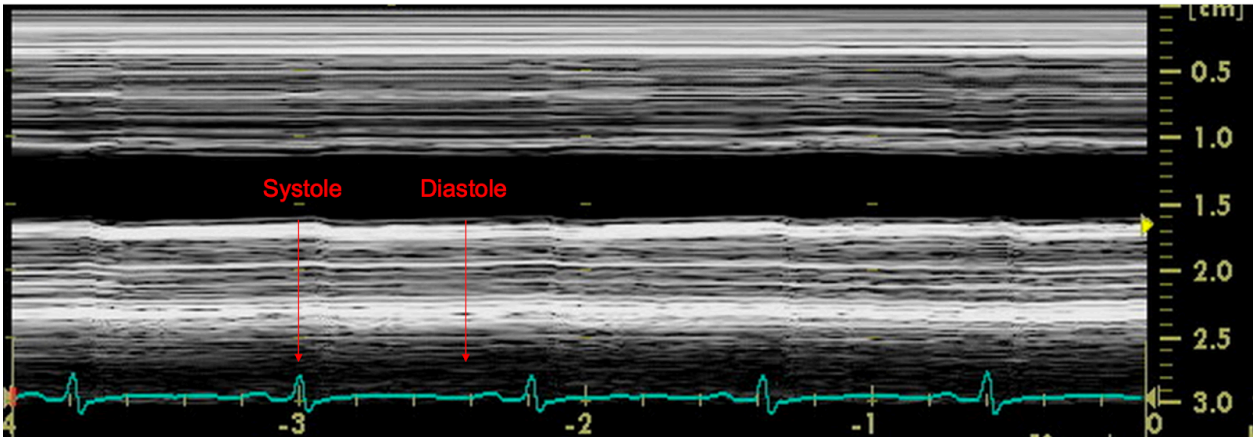

Figure 10. Vivid I ultrasound image of the brachial artery in M-mode, depicting changes in artery diameter relative to electrocardiogram heart rate cycles of systole and diastole.

1.13 Hypoxia and FMD

The impact of hypoxia bouts in BHD is difficult to predict. Hypoxia can impair endothelial function in healthy adults. On one hand, as endothelial cells are the major barrier between tissue and changes in the intravascular environment, they are the first to be exposed to environmental perturbations including reduced oxygen concentrations – yet are relatively resistant to such changes in oxygen (Pinsky et al., 1995). On the other hand, hypoxia and ischemia cause a repertoire of either adaptive or maladaptive responses to a stress stimulus termed “endothelial activation” and can result in changes in the endothelial barrier function, support of blood fluidity, cell growth regulation and modulation of vascular tone. Of particular interest as it relates to FMD is hypoxia and endothelial modulation of vascular tone. Acute introduction of mild hypoxia causes rapid elaboration of vasoconstricting agents such as endothelin-1. Considering the vasoconstrictor properties of endothelin-1, its excretion by the endothelial cells in hypoxia or ischemia can indicate dysfunction of the endothelium. Chronic exposure to oxygen deprivation, however, stimulates platelet-derived growth factor-B, leading to remodeling of the vessel wall and vascular smooth muscle cell proliferation (Ten & Pinsky, 2002), which ultimately could influence overall endothelial function.

We know that endothelium-derived NO plays a major role in the regulation of blood flow to various organs and smooth muscle cells, and is produced by eNOS (Janssens, Shimouchi, Quertermous, Bloch, & Bloch, 1992). Additionally ,eNOS has a profound effect on blood vessel tone by modulating the amount of NO delivered locally to specific organs in the body. Aberrations in the amount of NO produced can have deleterious effects, where elevated concentrations of NO are associated with conditions such as septic shock (Broner et al., 1993) and reduced quantities are associated with a number of vascular disorders such as atherosclerosis (Hayashi, Fukuto, Ignarro, & Chaudhuri, 1992), diabetes (Calver, Collier, & Valiance, 1992) and pulmonary hypertension (Archer, Tolins, Raij, & Weir, 1989). Acute hypoxia does not directly alter the generation of NO by endothelial cells; however, chronic exposure to hypoxia (>24 hours) suppresses the enzyme eNOS and decreases the release of NO production by the endothelial cells. Therefore, prolonged hypoxia reduces NO production, potentially impairing it's vasodilatory response to a shear stress stimulus (McQuillan, Leung, Marsden, Kostyk, & Kourembanas, 2017).

Although our understanding of hypoxia on endothelial function is clear, this relationship in divers remains equivocal. To draw conclusions on the physiology of BHD, we rely on studies evaluating endothelial function in SCUBA divers or athletes training at high altitudes. Specifically, acute and passive bouts of hypoxia through high-altitude exposure impaired FMD responses (Lewis et al., 2014), yet others found no change pre- and post-high-altitude hypoxia (Frick et al., 2006; Iglesias et al., 2015). The disparity in the findings could be due to the elevated/hypoxic state (576 m above sea level) during which pre- and post- measurements of FMD were taken (Frick et al., 2006), and the duration of the hypoxia exposure (4 hours) not being sufficient to be classified as chronic hypoxia (Iglesias et al., 2015). In SCUBA divers, FMD was reported to decrease significantly from 8.6 ± 1.3 % to 3.0 ± 0.8 % after a singular dive (Obad et al., 2010). Subsequently, a reduction in FMD was observed after a 20 minute SCUBA dive (Theunissen et al., 2013). The reason for the discrepancy in these studies may include differences in cuff duration for the FMD protocol, type of diver, control groups, shear stimulus, duration of training etc. Although SCUBA and high-altitude population groups are similar in nature to BHD, there are additional mechanisms involved which stop us from drawing generalized

conclusions from SCUBA and high altitude athletes, about BHD. For example, reductions in FMD are associated with increased concentrations of circulating NO in dynamic breath-hold divers mainly due to more physical exertion; whereas in scuba diving, those quantities decrease as NO reacts with superoxide anion O_2^- (produced by hypoxia)(Jamieson, Chance, Cadenas, & Boveris, 1986) to produce $ONOO^-$ (Sureda et al., 2009). Therefore, uncertainty remains regarding whether repeated bouts of hypoxia and/or long exposures under water affect endothelial function. This project addresses this knowledge gap by measuring the chronic baseline FMD in professional divers in the absence of an apnea to examine the vascular effects of chronic exposure to BHD, with quantification of the shear stimulus and by including an age-, and sex-matched control group. Thus, the FMD protocol will improve our understanding of the impact of breath-hold diving on endothelial function.

1.14 Purpose Question and Hypothesis

1.14.1 Purpose

The purpose of this project was to provide new insight into the vulnerability of endothelial function via flow-mediated dilation, in professional breath-hold divers compared to healthy controls.

1.14.2 Hypothesis

We tested the hypothesis that repetitive bouts of BHD will affect the endothelial function of professional breath-hold divers.

2 Introduction

Interest in breath-hold diving (BHD; or diving apnea) originated in the ancient Greek mythological traditions of the Ama over 2000 years ago, whose people dove to catch fish off the coast of Japan and Korea for 30-90 s bouts, up to 4 hours a day (Hurford et al., 1990). In the last 30 years, pushing the human limits of volitional breath-hold has become an organized sport. Professional BHD has branched into categories of static (non-exercising), dynamic (exercising) and deep sea (depth) diving. During each type of dive, athletes push themselves to states of severe hypoxia, defined as the deficiency of oxygen supply to an organ. In this thesis, we focused on static BHD.

The brain has a high metabolic demand using 20% of the body's energy stores, yet is unable to store oxygen itself. Thus, the brain requires constant regulation to maintain adequate oxygen delivery to endure normal functioning (Hoiland, Bain, Rieger, Bailey, & Ainslie, 2016). Elite divers meet oxygen demands during BHD through two fundamental principles: 1) augmented oxygen storage capacity (i.e. increased lung capacity), and 2) oxygen conservation. The latter is achieved through vagally-mediated bradycardia and sympathetically-mediated splenic and peripheral vasoconstriction (Foster & Sheel, 2005; Erika Schagatay, 2009). Through those mechanisms, apneists can prioritize oxygen-rich blood flow through the body, while simultaneously attenuating the decline in arterial oxygen saturation (Espersen et al., 2002).

Unsurprisingly, BHD has been linked to a series of acute dangers, including glossopharyngeal insufflation (Chung et al., 2010; Mijacika & Dujic, 2016), barotrauma, syncope, drowning (Lindholm & Lundgren, 2009), and cardiac arrest (Hong, Song, Kim, & Suh, 1967). Long-term health impairments continue to remain understudied in the BHD population.

An area of particular interest with regards to long-term impairments in BHD, is the integrity or health of the vascular endothelium. The endothelium has many physiological and pathological roles including regulation of smooth muscle tone, control of thrombosis, inhibition of leukocyte and platelet cell adhesion, and promotion of intra-arterial permeability (Celermajer, 1997; Rubanyi, 1993; Vane et al., 1990). Additionally, the

endothelium releases many vasoactive compounds including nitric oxide (NO) (Furchgott & Zawadzki, 1998), responsible for regulating diameter, tone and structure of the vasculature. Nitric oxide is the major contributor to vasodilation of vessels (Singel & Stamler, 2005). The endothelial cells contain shear stress-sensitive ion channels (Cooke, Rossitch, Andon, Loscalzo, & Dzau, 1991; Lansman, Hallam, & Rink, 1987; Olesen, Clapham, & Davies, 1988), and in response to increases in blood flow post supra-systolic ischemic release, these channels release NO (Moncada, Radomski, & Palmer, 1988), which causes vasorelaxation of the smooth muscle, and a subsequent increase in diameter.

Flow-mediated dilation (FMD) was first proposed as a reactive hyperemia endothelial function test by Celermajer and colleagues (Celermajer et al., 1992). Following a period of limb ischemia via pressure cuff, FMD is defined as conduit artery vasodilation in response to the increased internal vascular wall shear stress. Upon cuff release, the increased inflow of blood into the artery creates a moment of peak reactive hyperemia which translates to increased internal vascular wall shear stress and thereby constitutes the stimulus required to elicit FMD. Ultrasound is used to measure brachial artery diameter and blood velocity, and arterial blood flow is calculated as a product of both measures. The ultrasonic assessment of FMD in response to hyperemia is now established as a reliable, non-invasive measurement of endothelial function (Uehata et al., 1997). Peak reactive hyperemia (PRH) is defined as the maximal blood velocity peak as a result of the ischemia. PRH typically occurs within ~4-7 s post-cuff release, and returns to baseline within two minutes (Corretti et al., 2002). PRH is an indicator of microvascular health, yet no studies have examined the effect of chronic BHD training on downstream microvasculature.

Clinically, FMD is used to assess endothelial dysfunction, and lends utility when examining underlying acute or chronic stimuli that alter vascular function and risk (Ghiadoni, 2001; Karatzis et al., 2006). Hypoxia, as performed repeatedly by elite apneists, can impair endothelial function in healthy adults. The FMD technique is therefore a tool that improves our physiological insight and understanding of the mechanisms that alter both vascular and endothelial function in this BHD population.

Professional breath-hold divers are chronically exposed to bouts of hypoxia. Hypoxia and ischemia cause a series of either adaptive or maladaptive responses to a stress stimulus, and can result in changes in endothelial barrier function, support of blood fluidity, cell growth regulation and modulation of vascular tone. Acute introduction of mild hypoxia is associated with secretions of vasoconstricting agent endothelin-1; whereas chronic exposure leads to remodeling of the vessel wall, and vascular smooth muscle cell proliferation (Ten & Pinsky, 2002).

To draw conclusions on the physiology of BHD, we rely on studies evaluating endothelial function in SCUBA divers or athletes training at high altitudes. Specifically, acute and passive bouts of hypoxia through high-altitude exposure impaired FMD responses (Lewis et al., 2014), yet others found no change pre- and post-high-altitude hypoxia (Frick et al., 2006; Iglesias et al., 2015). In SCUBA divers, FMD decreased after a singular dive (Obad et al., 2010). These population groups however, do not properly represent the BHD group – as other mechanisms are involved in SCUBA and high-altitude individuals. For example, reductions in FMD are associated with increased concentrations of circulating NO in breath-hold divers due to more physical exertion; whereas in SCUBA diving, those quantities decrease as NO reacts to produce $ONOO^-$ (Sureda et al., 2009). Thus, uncertainty remains regarding whether repeated bouts of hypoxia and/or long exposures under water affect endothelial function in divers.

Therefore, this project addressed this knowledge gap by measuring the chronic baseline FMD in professional BHD in the absence of an apnea to examine the vascular effects of chronic exposure to breath-hold diving, with quantification of the shear stimulus and by including an age-, and sex-matched control group. The objective of this study was to provide new insight into the vulnerability of endothelial function via FMD, in professional breath hold divers. We expected protection of the brain and heart during hypoxia, but what happened at the peripheries during diving events was vastly understudied. We were testing the hypothesis that BHD would affect endothelial function in professional BHD, compared to healthy age-matched controls.

2 Methods

All testing was conducted in accordance with the Declaration of Helsinki and study ethics approval was obtained from the Research Ethics Board at Western University. Informed consent was acquired. Written informed consent was obtained from participants prior to participation. The study was approved at the University of Western Ontario's Human Studies Research Ethics Board for collection of data both in Novi Sad (111375) and London (107620), whose letters of information can be found in Appendix A and B. A health history screening and study eligibility were determined prior to experimentation.

2.1 Participants

Seventeen competitive breath-hold divers (31± 10 years; 3 females) from Split, Croatia and Novi Sad, Serbia were recruited through their respective training centers. Testing for divers occurred during peak-season in May 2018. All experimental participants travelled to Novi Sad for testing. The laboratory component of the testing took place at the human physiology laboratory in the Medical Faculty at the University of Novi Sad, Serbia. Seventeen healthy age- and sex-matched controls (32 ± 10 years; 3 females) were recruited from London, Canada. Testing in Canada took place at the Laboratory for Brain and Heart Health at Western University, London, Canada. Participant characteristics can be found in Table 1, and diving characteristics in Table 2.

Inclusion criteria included healthy individuals 18-65 years of age. Exclusion criteria included individuals with diabetes, impaired glucose tolerance, autonomic neuropathy, Parkinson's disease, history of psychosis, eating disorders, manic or bipolar disorder, major psychiatric conditions, dependence on alcohol or drugs within the past year, or severe traumatic brain damage. Individuals receiving pharmacological treatment that affects neural or vascular control such as psychiatric disorders, hypertension, increased cholesterol, heart disease or peripheral vascular disease (we may ask participants to come off medications for 2-3 days as appropriate prior to, and then during, testing) were also excluded. Participants were encouraged to eat and drink prior to testing; however, exercise, alcohol and caffeine were advised against 24 hours before testing. Of the six

female participants, four were in the follicular phase of their menstrual cycle, and two were in their luteal phase. Women who were pregnant or trying to become pregnant were excluded. Finally, participants were excluded if they were unable to provide written informed consent, or complete questionnaires or health history forms due to language or cognitive difficulties.

2.2 Protocol

Basic anthropometric measurements of participants were collected prior to the beginning of testing including: height (cm), weight (kg), age (years), heart rate (bpm), systolic, diastolic and mean arterial blood pressure (mmHg) for all participants (Table 1). Additionally, diving data from the experimental group regarding their years of experience, years of competitive BHD, training days per week, and duration of training can be found in Table 2. Blood pressures were recorded three times prior to testing, via an automated sphygmomanometry (BPM Model200, BpTru Medical Devices, Coquitlam, BC) and averaged. A medical screening questionnaire was administered to each participant to screen for medical conditions in the exclusion criteria.

Table 1. Participant characteristics of divers and controls.

Participant Characteristics	Divers	Controls
Total (n)	17	17
Sex	3F; 14M	3F; 14M
Age (years)	31 ± 10	32 ± 10
Height (cm)	182 ± 9	178 ± 6
Weight (kg)	81 ± 13	81 ± 14
Heart rate (bpm)	63 ± 9	66 ± 8
Systolic BP (mmHg)	116 ± 11	115 ± 9
Diastolic BP (mmHg)	71 ± 7	70 ± 8
Mean arterial BP (mmHg)	86 ± 8	85 ± 8

Values are mean ± SD. BP, blood pressure; M, male; F, female.

Table 2. Divers characteristics of seventeen participants.

Characteristics	Divers
Diving experience (years)	10 ± 9
Competitive apnea (years)	5 ± 4
Training per week (days)	5 ± 5
Duration of training (min)	70 ± 33
PR for static apnea (min:s)	5:19
Occasional forgetfulness (%)	30
Permanent forgetfulness (%)	20
Number of blackouts	5 ± 6

Values are mean ± SD. Percent (%) is the proportion of occurrences compared to the mean group. PR, personal record.

2.3 Initial Instrumentation

While supine, participants were instrumented with a 3-lead electrocardiogram (ECG; ADInstruments, Dunedin, Otago, NZ) for continuous measurements of heart rate (HR). A secondary 3-lead ECG, attached to a Vivid I ultrasound machine (GE Healthcare, Chicago, USA) provided ECG tracings during blood flow velocity (V) measurements of the brachial artery. Velocity measurements of the artery were taken using a 10-12 MHz probe (GE Healthcare, H40402LY, Chicago, USA) from the Vivid I machine, at an insonation angle of 60°. Manual adjustments to gain, steer angle, low frequency range and sample volume were made to find the clearest image of the brachial artery. A finger photoplysthmograph was placed on the middle phalange of the third finger to measure continuous beat-to-beat measurements of arterial blood pressure using a Finapres Finometer system (Finapres Medical Systems, Amsterdam, Netherlands), and that finger blood pressure was calibrated with an upper arm cuff. All devices were connected to a PowerLab A/D data acquisition system (ADInstruments, Dunedin, Otago, NZ) through

which analog data were sampled at 1000 Hz and saved for offline analysis using LabChart software (LabChart Pro v.8, ADInstruments, Dunedin, Otago, NZ).

A five-minute baseline period allowed for measurement of resting levels of all outcome variables. The Vivid I ultrasound machine was used to take baseline measurements of brachial artery diameter before beginning the pressor tests (Fig. 11).

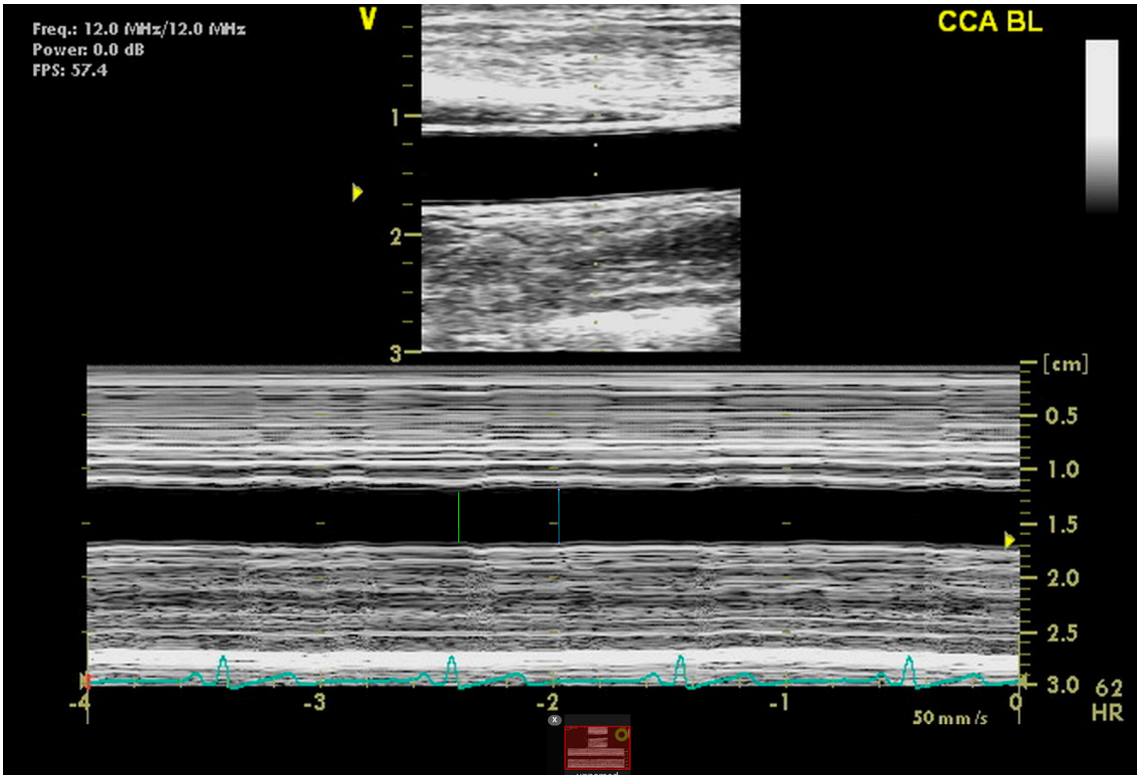

Figure 11. Depiction of Vivid I ultrasonography recording a baseline image of the brachial artery (Pulse-wave and M-mode).

2.4 FMD (Flow-mediated dilation)

Endothelial function was assessed by the Raitakari and Celermajer method (Raitakari & Celermajer, 2000). This method measures FMD and its respective reactive hyperemia (Corretti et al., 2002). A Hokanson cuff was placed one inch below the antecubital fossa on the participants' right arm (Hokanson, Model CC17, WA, USA). The ultrasound probe was placed over the brachial artery, just two to three inches above that same landmark (Fig. 8). A one-minute baseline period allowed time to capture three baseline

diameter measurements of the brachial artery (each fifteen seconds apart). Subsequently, the Hokanson cuff was inflated to ~50mmHg above the participants respective resting systolic blood pressure (or enough to stop radial artery pulse pressure) for five minutes. At minute six, the cuff was deflated, and images of brachial artery diameter were taken every fifteen seconds (three images); every five seconds (six images), then every fifteen seconds (seven images) to complete the nine-minute protocol (Fig. 12). Blood flow velocity (cm/s) was recorded by the Doppler ultrasound and output to the LabChart software. Ultrasound images were recorded as DICOM images and blood flow velocity as LabChart files and transferred to a computer for offline analysis.

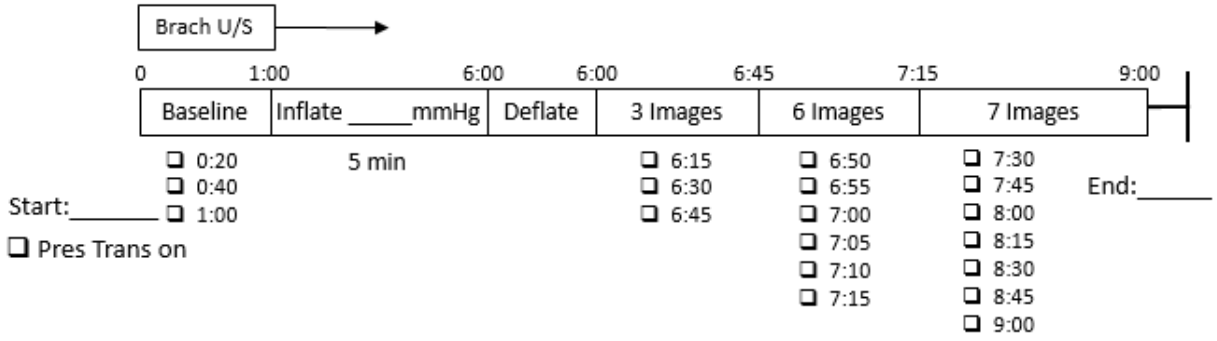

Figure 12. Schematic representation of the flow-mediated dilation protocol, indicating the timing of each image and the number of images taken. U/S, ultrasound.

2.5 Blinding

All ultrasonography files were blinded from the outcome assessor by an independent researcher, to reduce bias in vessel diameter measures. All images were measured three times, and an average diameter was collected. Files were only unblinded after all calculations had been completed, prior to statistical analysis. A pseudo-random selection of images were measured eights months apart to ensure agreement between measurements.

2.6 Calculations

Data was collected from the LabChart files and the Vivid I ultrasound images. Images were transferred to the Osirix software to allow manual measurements. Velocity data were then selected at ten second intervals prior to capturing images, as during images the velocity recording was stopped temporarily. Selection of the time point was based on the ten second period with the cleanest velocity data. Cross sectional area (CSA; cm^2) was calculated using brachial artery diameter manually collected from the Vivid I images (Fig. 3), on OsiriX MD (OsiriX, Bernex, Switzerland) as CSA = [$\pi(d/2)^2$], where d represents diastolic diameter (cm). Baseline diameter was averaged from the three time points, and peak diameter (cm) was the largest diameter measured 30 s post cuff deflation. Forearm flow was calculated with brachial artery CSA (cm^2) and velocity (cm/s) as F(mL/min) = CSA x velocity. Peak reactive hyperemia (cm/s) was calculated by selecting the maximal peak of brachial artery velocity during the first 20 seconds following cuff deflation (Fig. 6). FMD percent change (%) was calculated with peak diameter and baseline diameter of the brachial artery, where %FMD = {[(peak d – baseline d)/baseline d] x 100%}. Total shear (cm/s.s) was calculated by measuring the area under the velocity curve at the moment of cuff deflation until the end of the FMD protocol. Relative shear was calculated as relative shear = [individual %FMD / individual total shear].

3 Statistical Analysis

Our primary research outcomes were %FMD and PRH between the two participant groups. Secondary outcomes included HR, SBP, DBP, baseline diameter, total shear, and relative shear between participant groups.

A Bland-Altman test was used to evaluate the agreement among BA the two diameter measurements that were separated by eight months (Bland & Douglas, 1986) using SPSS Statistics (IBM SPSS Statistics 25, Armonk, NY, USA). Five diameter measures were made for each individual in a randomized format with measured obtained at baseline 1, 6:15, 6:55, 7:30 and 9:00 from the FMD beginning of the protocol. The measures were

obtained from five controls and five divers. The mean difference of the measurements was calculated and plotted as a horizontal line. The lower and upper limits of agreements (LOAs) were calculated as the mean difference ± 1.96 times the standard deviation.

3.1 Independent sample t-tests

Descriptive statistics were used to present participant characteristics and outcome data. Data for all outcomes are mean ± standard deviation. Data from the primary outcomes (i.e. %FMD, PRH) were reported as mean ± standard deviation along with 95% confidence interval (CI) and effect sizes. Effect sizes were calculated using Cohen's *d* for between group study designs and interpreted as small 0.2, medium 0.5 and large 0.8 (Fritz, Morris, & Richler, 2012). Normality distributions of each variable between divers and controls were similar, as assessed by visual inspection. Outliers were individually inspected, and were not removed from the data (Fig. 22). All outcomes for both diver and control groups were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$). There was homogeneity of variances for all outcomes in divers and controls, as assessed by Levene's test for equality of variances ($p > 0.05$). Independent sample t-test were run to examine any differences between divers and controls. Confidence intervals were interpreted for significant interactions.

3.2 Simple regression model

Four dependent variables were selected to compare to years of training in divers, for individual simple regression models: %FMD, PRH, baseline artery diameter and maximal BHD duration. There was independence of residuals, as assessed by a Durbin-Watson statistic of 2.109. There were no linear relationships found between %FMD (R^2=0.004), PRH (R^2=0.071), baseline artery diameter (R^2=0.027) and maximal BHD duration (R^2=0.037) and years of BHD training (Fig. 15-18).

4 Results

The Bland-Altman confirmed the agreement in measurements of BA diameter at different time points, where evenly distributed proportional bias was observed on either ends of the LOAs (p=0.620). No systematic bias was observed (p=0.422).

Although divers reported significantly longer breath-hold records, there was no significant difference in %FMD found between divers (7.46 ± 7.80%) and controls (5.30 ± 7.21%), M= 2.20, 95% CI [-3.10, 7.52], t(32)=0.845, p=0.404, d=0.3 (Fig. 13). Peak reactive hyperemia however, was statistically diminished in divers (124 ± 27.93cm/s) compared to controls (159 ± 34.31cm/s), M= -34.69, 95% CI [-56.55, -12.83], t(32)= -3.232, p= 0.003, d=1.1, power=0.89 (Fig. 14). Total shear (D: 3705 ± 1583 cm/s.s; C: 3537 ± 1136 cm/s.s) and relative shear (D: 0.002 ± 0.002 C: 0.002 ± 0.002) did not differ between groups (p > 0.05). Baseline artery diameter (D: 0.432 ± 0.068 cm, C: 0.442 ± 0.061 cm) SBP (D: 135 ± 13.69 mmHg, C: 131 ± 20.26 mmHg), DBP (D: 67.70 ± 7.30 mmHg, C: 74.17 ± 12.61 mmHg) and MAP (D: 81.52 ± 22.08 mmHg, C: 93.60 ± 14.39 mmHg) were not significantly different between groups (Table 3). Divers had significantly lower baseline HR (D: 55.00 ± 7.70 bpm, C: 60.91 ± 8.48 bpm) compared to controls (p < 0.05; Table 3). Raw data can be found in Appendix C.

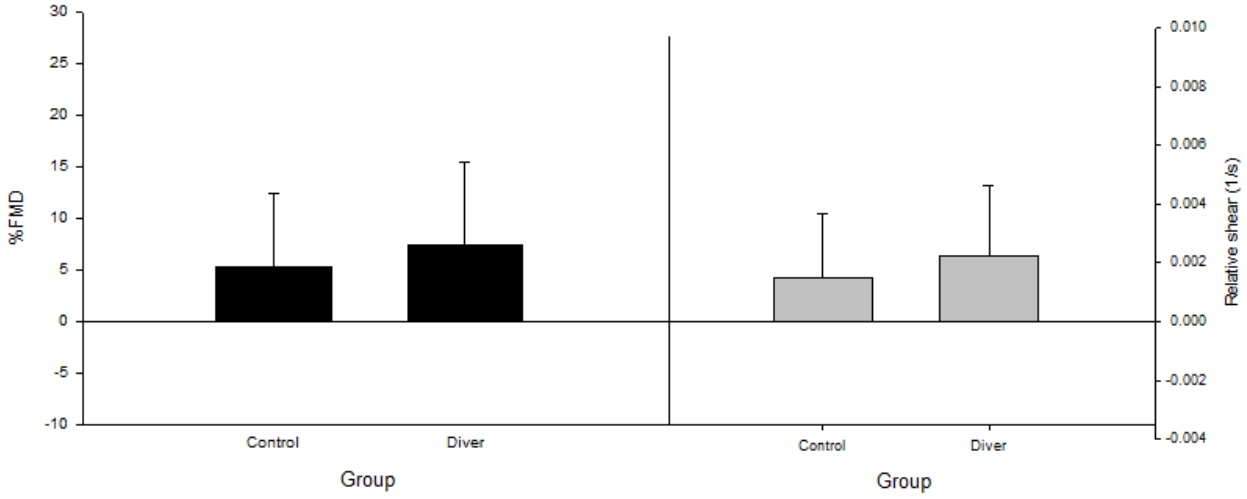

Figure 13. %FMD response, and relative shear (1/s) between divers and controls. FMD, flow-mediated dilation. No significant differences between divers and controls.

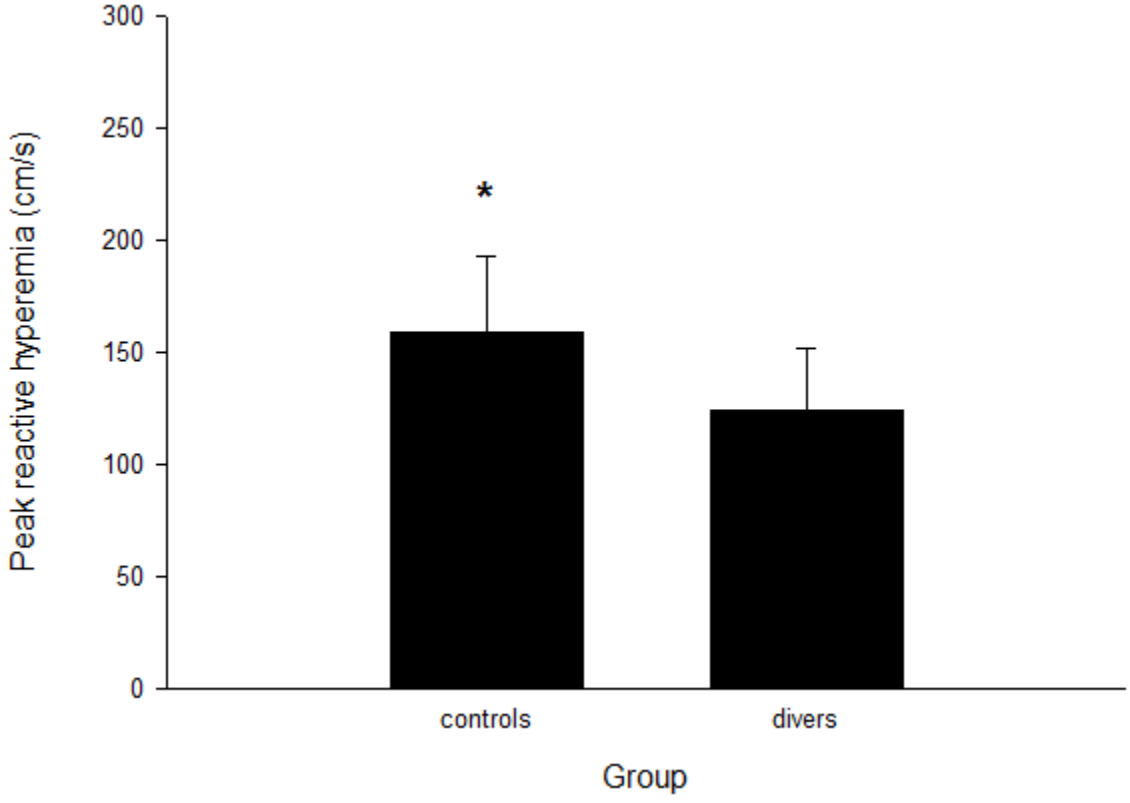

Figure 14. Peak reactive hyperemia response between divers and controls at cuff deflation. *Significantly greater response than divers ($p<0.05$).

Table 3. Results of secondary outcomes.

	Mean difference	95% CI [L;U]	*t* value	*p* value	cohen's *d*
Total shear	167.610	-794.78; 1123.00	0.355	0.725	0.1
Relative shear	0.001	-0.0008; 0.00238	0.994	0.327	0.4
BL BA diameter (cm)	-0.010	-0.05535; 0.03481	-0.464	0.646	0.2
HR (bpm) *	-5.600	-11.5732; -0.2411	-2.124	0.042	0.7
SBP (mmHg)	4.364	-8.0003; 16.7285	0.719	0.477	0.3
DBP (mmHg)	-6.469	-13.6682; 0.73103	-1.830	0.077	0.6
MAP (mmHg)	-12.076	-25.0966; 0.94411	-1.889	0.068	0.7

Values are mean. BL, baseline; BA, brachial artery; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; CI, confidence interval; L, lower; U, upper. * indicates significant difference between divers and controls ($p<0.05$).

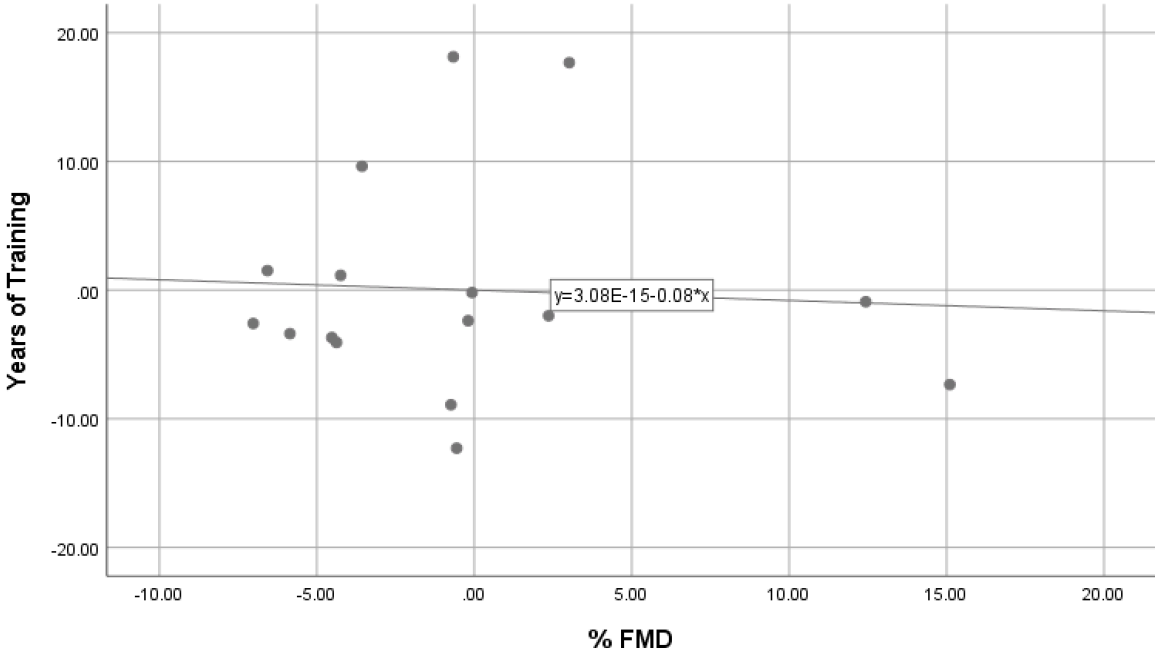

Figure 15. Schematic representation of linear regression model comparing % FMD (flow-mediated dilation), and years of BHD training (R^2=0.004).

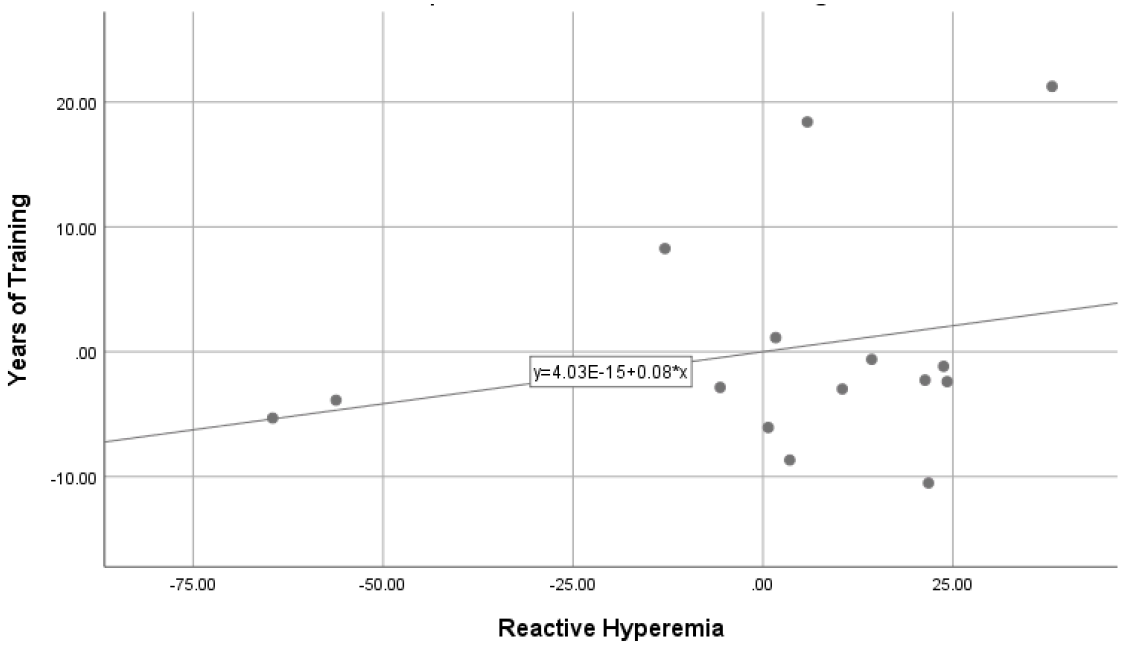

Figure 16. Schematic representation of linear regression model comparing peak reactive hyperemia, and years of BHD training (R^2=0.071).

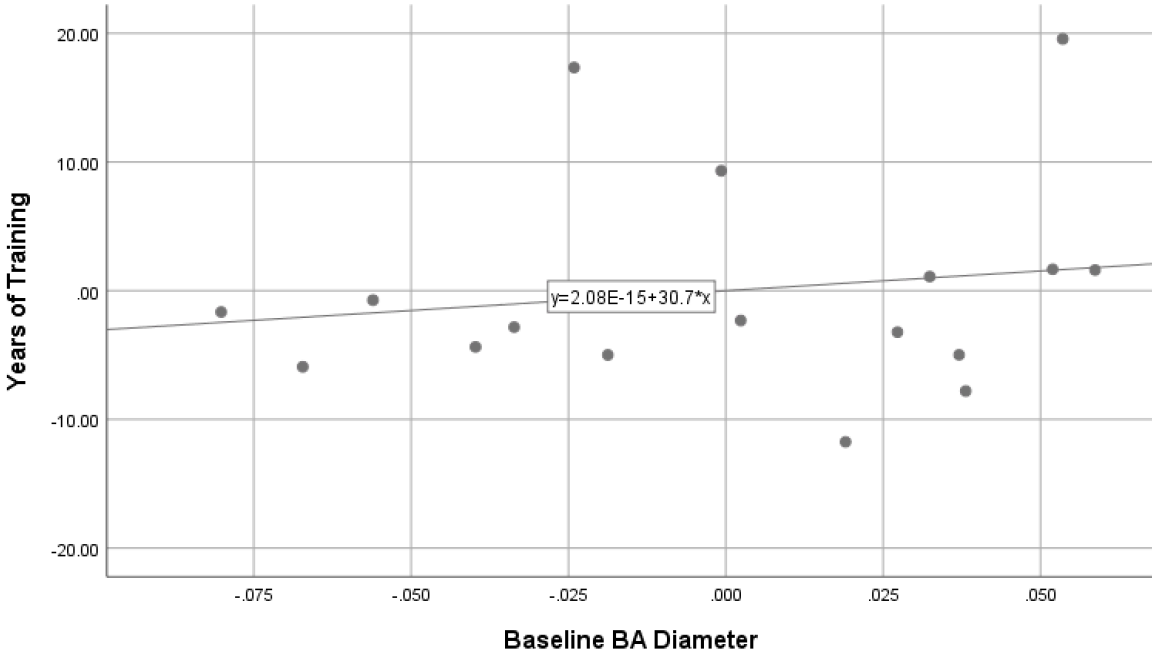

Figure 17. Schematic representation of linear regression model comparing baseline artery diameter, and years of BHD training (R^2=0.027). BA, brachial artery.

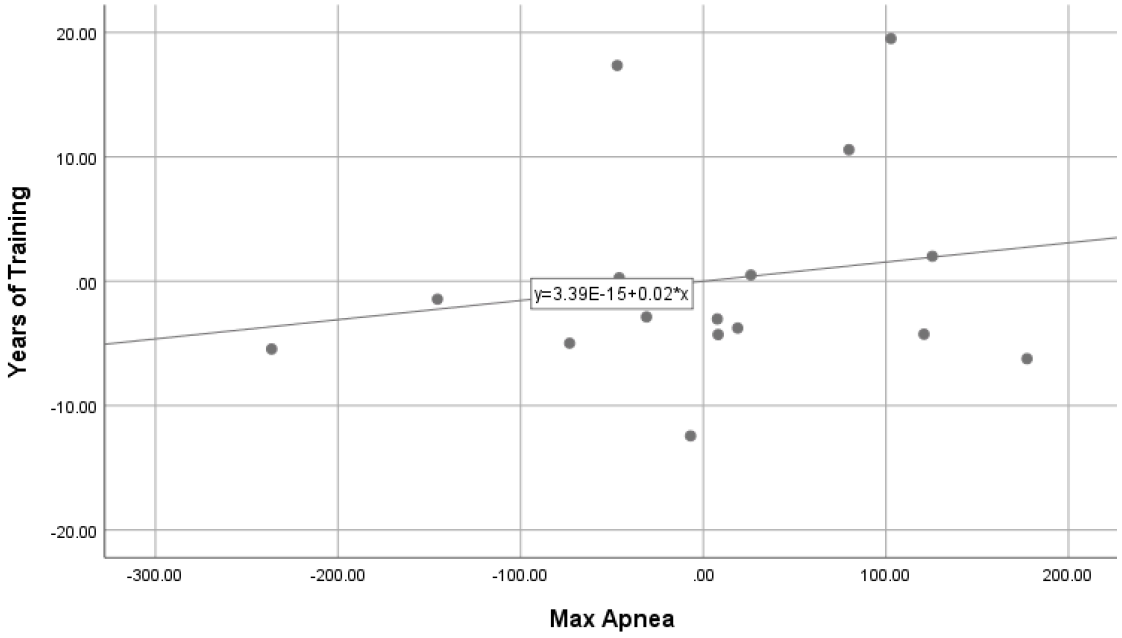

Figure 18. Schematic representation of linear regression model comparing maximal BHD duration, and years of BHD training (R^2=0.037).

Changes in artery diameter, blood flow velocity, and blood flow were measured over the entire FMD protocol and plotted in Figures 19-21.

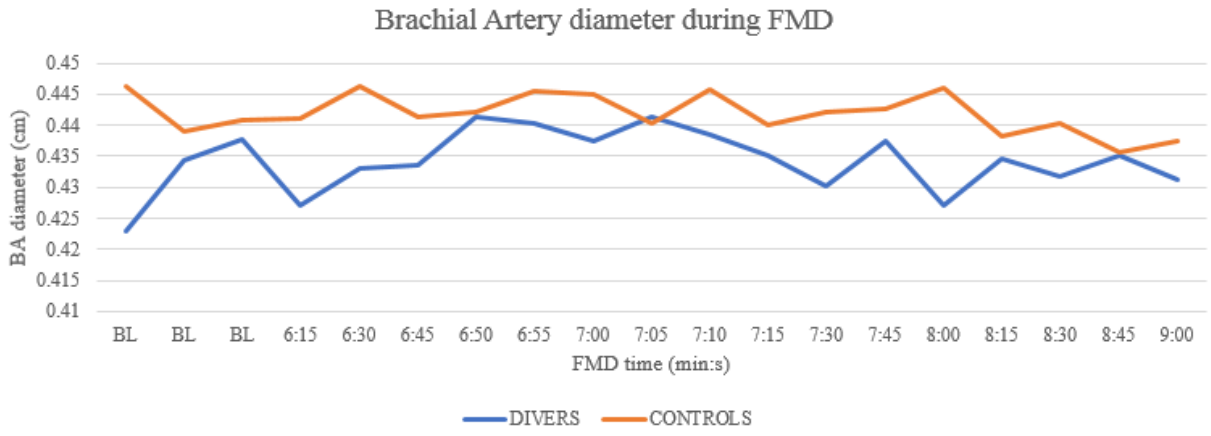

Figure 19. Differences in the changes in brachial artery diameter over the flow-mediated dilation protocol, between divers and controls. BA, brachial artery; BL, baseline; FMD, flow-mediated dilation.

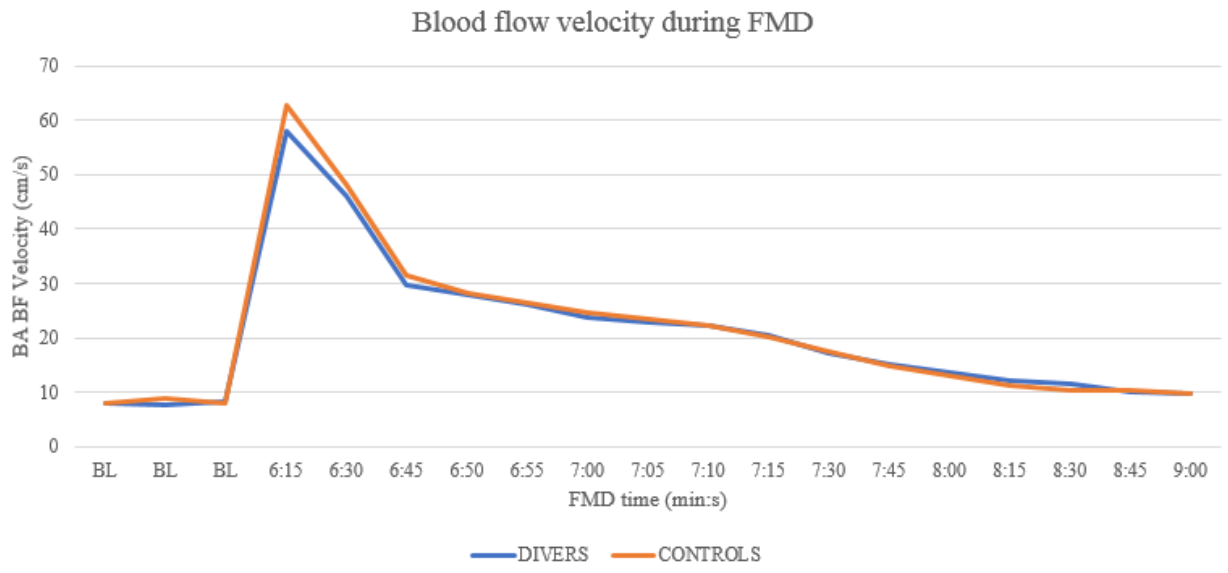

Figure 20. Differences in the changes in brachial artery blood flow velocity over the flow-mediated dilation protocol, between divers and controls. BA, brachial artery; BF, blood flow; BL, baseline; FMD, flow-mediated dilation.

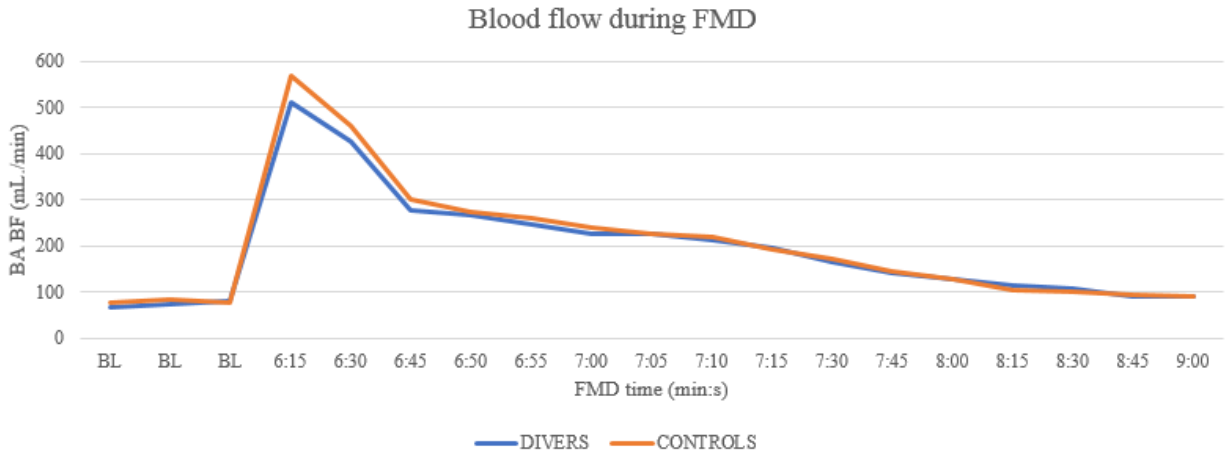

Figure 21. Differences in the changes in brachial artery blood flow over the flow-mediated dilation protocol, between divers and controls. BA, brachial artery; BF, blood flow; BL, baseline; FMD, flow-mediated dilation.

5 Discussion

5.1 Main findings

This study examined the endothelial function of competitive breath-hold divers by using an FMD experimental protocol. The FMD responses were similar between divers and healthy controls; thus, these data do not support our hypothesis that alterations in endothelial function, measured via FMD, occur in BHD. Although both groups received the same shear stimulus and had the same dilatory response, PRH was reduced in divers compared to controls. This was an unanticipated outcome, which may indicate differences in microvascular dilatory responses to ischemia.

5.2 Contextualizing findings within the literature

This current study found no difference in endothelial function via FMD responses between divers and controls. There is limited BHD literature with which to compare our current results; however, data from SCUBA, air dives and deep sea diving may be a helpful comparison. A study found a reduction in relative FMD in scuba divers (94.3 ± 7.3% of pre-dive FMD) and breath-hold divers (95.4 ± 3.5 % of pre-dive FMD) following 25 minutes of successive dives (Theunissen et al., 2013). This approach reports relative pre-post FMD, not providing a direct comparison to the values collected in our study. Additionally, Theunissens' paper looked at the effects of successive dives on FMD between SCUBA and BHD groups, where this thesis focuses on chronic resting state FMD characteristics, and not an acute post-dive response. Another study reported similar reductions in FMD response in experienced breath-hold divers immediately pre- and post- air dive in a hyperbaric chamber (9.2 ± 6.9 % to 5.0 ± 6.7 %) (Brubakk et al., 2005); however, this exposure was hyperoxic in nature, which makes it difficult to draw conclusions for comparison with the current study. Additionally, the aforementioned study reported acute changes in endothelial function, whereas our study sought to explore FMD after chronic (long-term training) exposure to BHD. A series of successive deep sea dives also displayed reductions in FMD after every dive, beginning at 8.6% prior to the first and 5.7% before the sixth dive (Obad et al., 2010), highlighting the acute endothelial

dysfunction associated with deep sea dives in experienced divers. The present data were not easily comparable to other studies on endothelial health in SCUBA divers, as they compared %FMD pre- and post-dive, or dynamic and/or deep sea diving as opposed to static BHD. Another difference between the SCUBA group and BHD, is that SCUBA divers do not reach hypoxic levels but rather they breathe hyperoxic gas concentrations. Hyperoxic gas mixtures can impair systems that require oxygen like eNOS (Pasgaard et al., 2007). Although, when examining the pre-dive FMD values, our current FMD data points were within the range seen in previous research. The comparison to deep sea diving was difficult due to its associated mechanisms not found in breath-hold dives, including transient loss of vascular homeostasis due to the bubbles causing direct injury to the vascular endothelium in both pigs (Nossum, Koteng, & Brubakk, 1999) and humans (Hjelde, Bergh, Brubakk, & Iversen, 1995). Due to differences in underlying mechanisms, it is difficult to draw conclusions of vascular health between sports on BHD. In summary, this study found that chronic bouts of static BHD did not show impairments in endothelial function.

5.3 FMD values

The range of FMD responses varied from 2.2 ± 2.4 % to 12.6 ± 6.7 % in healthy individuals (Clarkson et al., 1999; Neunteufl et al., 1997). A larger scale meta-analysis found the range of FMD values for 4,739 healthy adults free from cardiovascular disease ranged from -10.5 to 30.3% (Skaug et al., 2012), although they examined individuals ranging from 20-70+ years, and comorbidities may have influenced those extensive ranges (additional data provided by authors). Another study had found FMD responses of 10.5% in healthy controls and 6.3% in patients with atherosclerosis (Kuvin et al., 2001). Contrarily, FMD values in our current study were not different than the healthy controls (D: 7.46 ± 7.80% vs. C: 5.30 ± 7.21%), although no participants reported any abnormal medical history. The differences in FMD ranges between studies can be potentially attributed to comorbidities and age differences.

5.4 Differences in PRH

Temporary occlusion of circulation to a limb, followed by a rapid augmentation in blood flow following removal of the occlusion, is referred to as reactive hyperemia. In the FMD protocol, this reactive hyperemia is what initiates the shear stress that causes the large conduit artery to dilate. However, the cause of the reactive hyperemia comes from further down in the vascular bed. In particular, dilation induced by the ischemia is suddenly exposed to the flow upon removal of the occlusion leading to a very large and rapid pressure gradient that elicits the large flow response. Thus, the stimulus for the reactive hyperemia is the downstream dilation that occurs during ischemia (Pyke & Tschakovsky, 2005). This type of model is often used to explore measures of downstream dilation for a given period of ischemia. The divers in this study showed a reduction in PRH when compared to controls. This suggested a smaller dilatory response to the ischemic stimulus.

A study investigating % change in hyperemia, found that patients with obstructive sleep apnea had greater changes in blood flow velocity (an analog of blood flow) (405 ± 53%) compared to obese control subjects (375 ± 61%)(Kato et al., 2000). This earlier study, however, did not include a control group, thus interpretation of results should be considered carefully. As previously mentioned, the PRH stimulus involves a myogenically mediated dilation where the moment of cuff release causes dilation in the vessel to match the downstream dilation that was occurring during occlusion. In order to activate the metabolic components of PRH, a contractile tool would have been involved such as an isometric handgrip.

The mechanism(s) mediating the impaired PRH in BHD are not known but may involve an effect of repeated insults of prolonged hypoxia on the microvasculature. Specifically, a robust angiogenesis response occurs following a period of ischemia. This neovascularization combined with the opening of collateral vessels is able to restore blood flow to the once compromised muscle (Couffinhal et al., 1998; Limbourg et al., 2009). A highly organized network of arterioles, capillaries and venules are arranged to optimize oxygen transport, and requires the necessary conduits for red blood cells (RBC)

to deliver their oxygen and meet the metabolic demands of the tissues. An important requirement for this network is local oxygen content, which informs the arterioles to vasoconstrict or vasodilate as appropriate (Segal, 2005). If these networks are altered (i.e. via hypoxia), the regeneration of new microvascular networks will take place. A study examining reconstruction of microvasculature in >8 minutes of hindlimb ischemic and hypoxic mice found that the regenerated microvasculature displayed aberrant arteriolar branching, slow RBC transit through capillaries, impaired vasomotor control, arteriolar-venular shunting, and a monotony of RBC transit velocities through the capillaries. Further, this flawed regenerated microvascular network was still evident four months after ischemia, highlighting the failure to normalize (Arpino et al., 2017). Therefore, we speculate that the microvasculature of BHD may be impaired following chronic repeated exposure to hypoxia.

5.5 Limitations

5.5.1 Large ranges of FMD values

This study was not without its limitations. To begin, there were large ranges of % FMD responses in divers (-2.83 to 27.83 %) as well as in controls (-6.64 to 22.98 %)(Appendix C). When schematically presented on a box plot, three data points were flagged as extreme outliers (two in divers, one control; Fig. 22); however, these are within the ranges presented in the Skaug study (Skaug et al., 2012). The negative FMD responses could be interpreted as constrictor patterns, and although not typically associated with healthy outcomes, they do occur in healthy individuals. Data were blinded and re-analyzed three times to ensure no methodological issues accounted for the outliers.

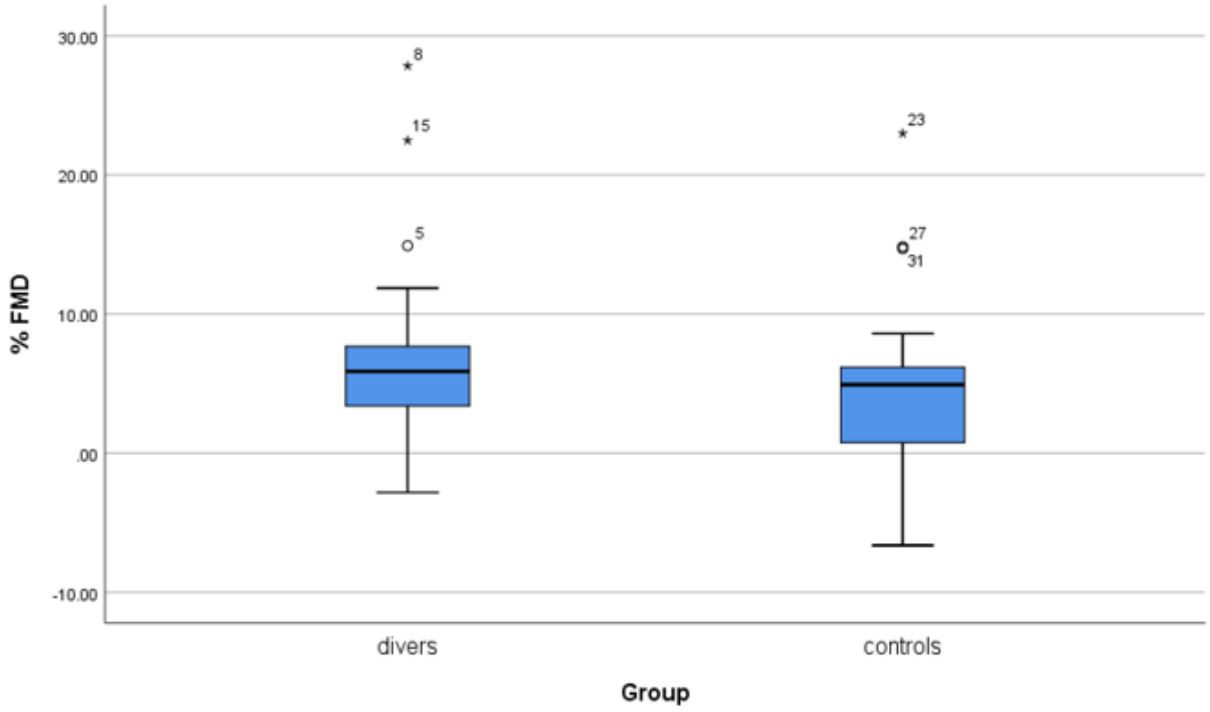

Figure 22. Boxplot of the range of % FMD data in divers and controls. Circular dots represent outliers as 1.5 box-lengths from the edge of the box, stars signify extreme outliers as 3.0 box-lengths from the edge of the box. FMD, flow-mediated dilation.

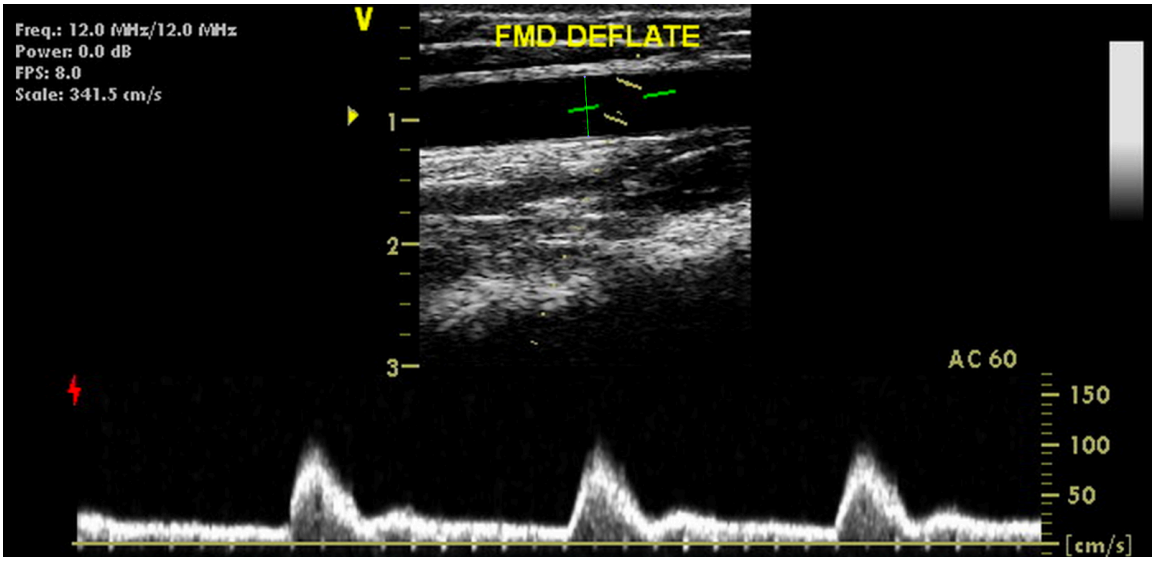

Figure 23. Ultrasound image viewed offline in OsiriX, depicting both the clean brachial artery diameter (top) as well as blood flow velocity profiles during FMD deflation (bottom). FMD, flow-mediated dilation.

5.5.2 Volume of the arm

Secondly, differences in FMD response could have influenced by the size of the arm. Our study did not account for the individual measurement of arm volume; however, no significant difference in body mass was detected between groups.

5.5.3 Occluded blood flow velocity values

Following, during the occlusion phase of FMD, the blood flow velocity did not completely reach 0 cm/s. This can be explained by the placement of the Hokanson cuff approximately one inch below the antecubital fossa, and the ultrasound probe placed two to three inches above that same landmark, over the brachial artery. As the probe was measuring velocity above the occluded area, there was still some blood flowing through the artery before reaching the moment of full occlusion of the cuff. However, there was also a large negative flow during diastole. The pattern indicates that flow moved forward and backwards in the artery with very little net forward flow.

5.5.4 Location of laboratory testing

As participant groups were measured in two laboratories, there were alternate methods to collect blood pressure; however, no differences were detected between groups in any blood pressure outcome (SBP, DBP, MAP; Table 3). In Canada, a forearm cuff was placed on the participants arm to calibrate finger BP; however, a formula was used to get the same calibration in Serbia.

5.5.5 Menstrual cycle stage monitoring

Lastly, there was no standardization of time of menstrual cycle in the female participants, although previous research has found that it is ideal to collect data during the first 1-7 days of the menstrual cycle to avoid the effect of hormones. Data was collected when female divers were available, due to the two-week time limitation of the data collection period in Serbia. Coincidentally, four of the six females were in their follicular menstrual phase, in which hormones are the lowest.

5.6 Benefits

5.6.1 Control group

An advantage of this study was the control group. Unlike many studies, this study age- and sex-matched all 17 participants in the experimental group in Serbia to an individual in Canada with the same participant characteristics (e.g. sex and age).

5.6.2 Controlled shear stimulus

FMD has been studied using a variety of approaches, which may influence the outcome. The magnitude of the shear stimulus created with reactive hyperemia is largely influenced by several factors (i.e. sex, age) and can vary substantially between participants and groups (Herrington et al., 2001; Joannides et al., 2002). Differences in occlusion durations, cuff position (lower vs. upper arm), and area of artery examination all result in distinct shear stress profiles, thus making it hard to compare findings between studies. Most researchers use normalization techniques to reduce the variability between participants due to differences in the magnitude of the shear stress stimulus. Shear rate is therefore considered the appropriate normalizing factor (Pyke & Tschakovsky, 2005). Normalization from the initial 60 s following cuff release (Pyke & Tschakovsky, 2007), or the time from post occlusion to peak artery diameter (Black et al., 2008; Padilla et al., 2008), are thought to be the best alternatives to eliminate the impact of stimulus variability between participants. Considering the importance of reducing variability in the shear stress stimulus administered to both of our participant groups, we measured total shear rate AUC and compared between groups. Both divers and controls received the same shear stimulus. Further, when dividing each %FMD response by their respective total shear, we found no difference in relative shear either. This indicates that the differences between groups were not due to variability in stimulus administration.

5.6.3 Cuff placement

Studies have variably used upper arm and forearm cuff occlusion, and it remains unclear whether one technique is more accurate or precise than the other. A study found that cuff

placement below the elbow resulted in 36% reduction in FMD, and 30% reduction in reactive hyperemia compared to above the elbow. Additionally, cuff placement below the elbow is associated with no evidence of sympathetic activation, unlike above the elbow (Mannion et al., 1998). Another study who administered an NO blockade at both distal and proximal cuff locations, found that when the cuff was above the elbow, the FMD response (12%) was only partially decreased by NO blockade compared to the 7% FMD response with cuff below the elbow (Doshi et al., 2001). Due to the effect of dilators other than NO and myogenic responses on the dilation of arteries within the ischemic territory, the recommendation is having cuff occlusion below the imaged artery to ensure a vasodilator response on the endothelium. In conclusion, cuff placement below the elbow was found more favorable for large-scale populations studies in regards to comfort and feasibility of the technique, compared to above.

5.7 Implications

This study was unique as it is currently the only study examining the effect of years of BHD training on resting endothelial function. Although the acute health risks are familiarly presented in research, the long-term health risks of frequent maximal BHD remain understudied. Studies have discovered that measures of microvascular function directly correlate with reactive hyperemia and could provide insight for cardiovascular risk prediction greater than FMD (Mitchell et al., 2004). Although brachial artery FMD has emerging as an independent predictor of future cardiac events (Gokce et al., 2002), it was found that FMD is only related to low and not medium nor high cardiovascular risk (Witte et al., 2005). In a group of 1,477 men without cardiovascular disease, blood velocity and shear stress during reactive hyperemia had a stronger association with cardiovascular factors than FMD (Philpott et al., 2009). Additionally, hyperemic velocity, and not FMD, was found to be an independent predictor of adverse outcomes in 1,574 healthy individuals (Anderson et al., 2011). In regards to implementation to clinical practice, the PRH outcome has the potential to provide insight into the microvascular health of both healthy and at risk populations. Future research should investigate the reconstructed microvasculature in breath-hold divers compared to controls, to examine

the differences in neonetwork architecture, and whether this new microvasculature is responsible for the attenuated PRH responses in divers.

5.8 Conclusion

Endothelial function was found to be unaltered in professional breath-hold divers, contrary to what we had originally hypothesized. The attenuated PRH responses in divers was potentially caused by ill-suited reconstructed microvasculature downstream, due to chronic exposure to hypoxia. Despite the limitations, this study provided insight into the endothelial and microvascular health of professional breath-hold divers. Based on the findings in this study, we concluded that long-term diving did not impair endothelial health in divers, yet it may impair downstream microvasculature.

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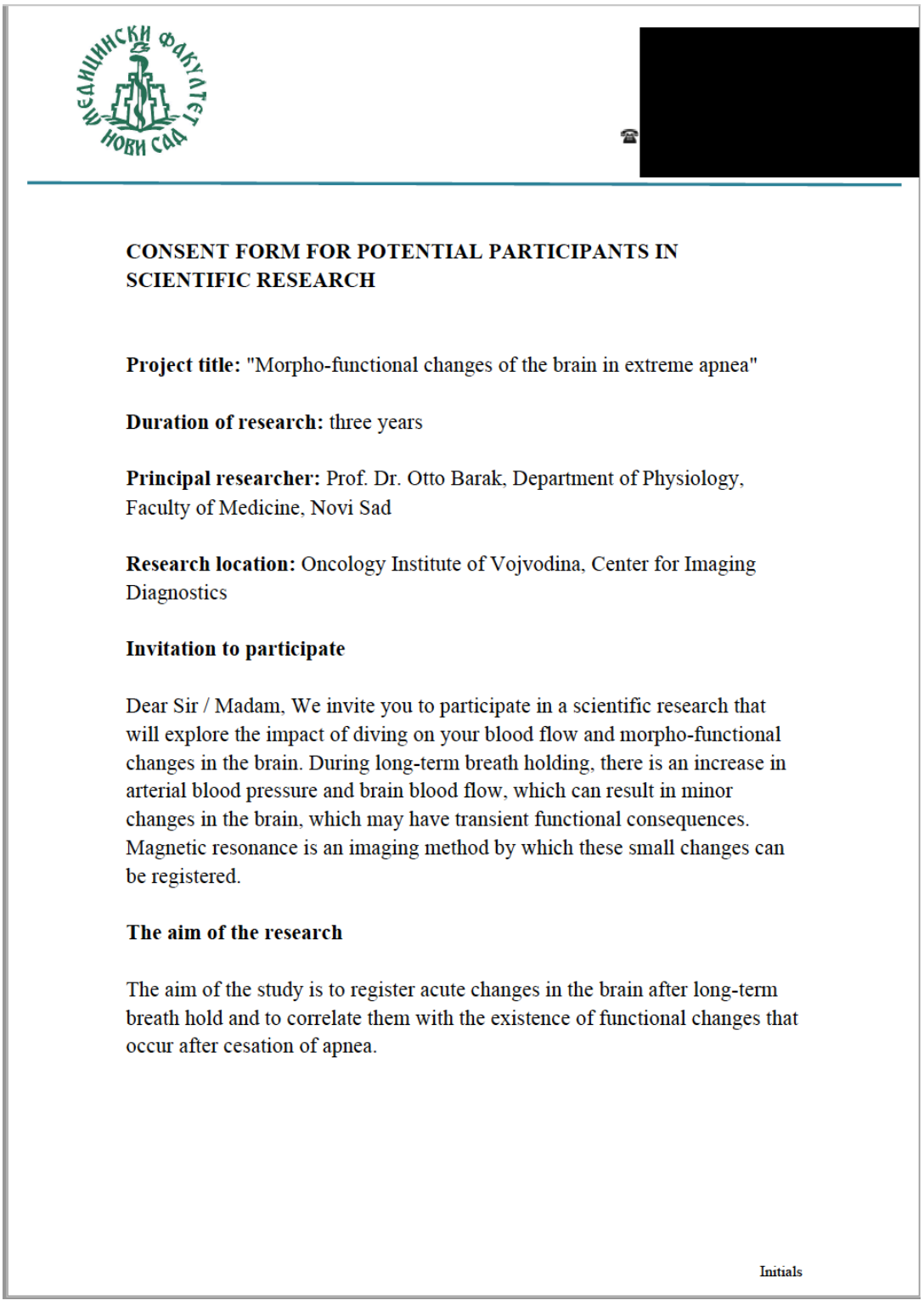
Appendices

Appendix 1: Letter of Information BREATHE Ethics #111375



МЕДИЦИНСКИ ФАКУЛТЕТ НОВИ САД

[redacted]

CONSENT FORM FOR POTENTIAL PARTICIPANTS IN SCIENTIFIC RESEARCH

Project title: "Morpho-functional changes of the brain in extreme apnea"

Duration of research: three years

Principal researcher: Prof. Dr. Otto Barak, Department of Physiology, Faculty of Medicine, Novi Sad

Research location: Oncology Institute of Vojvodina, Center for Imaging Diagnostics

Invitation to participate

Dear Sir / Madam, We invite you to participate in a scientific research that will explore the impact of diving on your blood flow and morpho-functional changes in the brain. During long-term breath holding, there is an increase in arterial blood pressure and brain blood flow, which can result in minor changes in the brain, which may have transient functional consequences. Magnetic resonance is an imaging method by which these small changes can be registered.

The aim of the research

The aim of the study is to register acute changes in the brain after long-term breath hold and to correlate them with the existence of functional changes that occur after cesation of apnea.

Initials

[redacted]

Participants

We intend to involve about 15 young divers with short experience and 15 older divers with longer experience in breath hold diving.

Procedure

During the research, you will be subjected to magnetic resonance imaging. When arriving at the lab, you will be informed in detail about the conditions under which the test will be carried out. At the beginning, you will lie steadily in the magnetic resonance device, and during that period doctors will take the images of your brain. You will then be asked to keep your breath as long as you can (as during your training), and images of your brain will be acquired after the cessation of the apnea.

What are the benefits of participation for you as respondents?

Participation in the research does not bring you any financial gain. The results of this study will additionally increase the knowledge of processes occurring in the brain during extreme apnea. In this way, we want to increase safety during diving for you and everyone involved in this sport. Upon completion of the research, you will be given insight into your findings and all new knowledge gained during the research.

What are the possible risks of participating in this research?

Magnetic resonance is a non-invasive method that does not impose any health risks for participant. During the recording, you will find yourself in a cramped space, which may cause discomfort to some.

Is participation compulsory?

Initials

You decide wether you want to participate in the research. If you choose to participate, sign your initials on each page of this consent form. Participation in this research is voluntary and you can withdraw from it at any time without the need to state the reasons. For any additional questions, you can contact the Principal Researcher, both before and during the course of the research.

Confidentiality and insight into documentation

When accessing this scientific research, we will give you an identification number that will protect your identity. The documentation may be reviewed by the Principal investigator, lead researchers and members of the research team as well as representatives of the relevant Ethics Committee.

Initials

☎ [redacted]

COMPLIANCE – INFORMED CONSENT OF PARTICIPANT

Project title: "Morpho-functional changes of the brain in extreme apnea"

Principal researcher: Prof. Dr. Otto Barak, Department of Physiology, Faculty of Medicine, Novi Sad

1. I confirm that I have received, read, and understood the document on the above stated research.
2. I confirm that the research has been explained to me in detail both in written form and verbally. I know to whom to address in case of possible problems.
3. I know that my participation in this scientific research is voluntary and that I can retret from it at any time without any consequences to my health or any legal consequences.
4. I understand that my documentation is under open access to responsible individuals, i.e. Principal investigator, lead researchers and members of the research team, as well as representatives of the relevant Ethics Committee. I give permission to these individuals to access my documentation.
5. I want to participate in the above stated scientific research.

Name and last name of participant	Signature	Date
Name and last name of researcher	Signature	Date

Initials

Appendix 2: Letter of Information for Ischemic Heart Disease Ethics #107620

Western HealthSciences

School of Kinesiology

Letter of Information

TITLE: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation: Control Group

Principal Investigator: Dr. Kevin Shoemaker
Research Staff: Jen Vording, Mark Badrov, Arlene Fleischhauer, Jeff Risdon, Baraa Al-Khazraji, Peter Prior, Neville Suskin.
Sponsor: Canadian Institutes of Health Research

INTRODUCTION AND PURPOSE

You are being invited to participate in a research study that will examine the role of vascular disease on the size and function of the brain and the health of blood vessels in the brain. We are particularly interested in how vascular disease affects brain blood flow as well as whether or not exercise training improves brain blood flow in individuals with, or at risk for, cardiovascular disease. The study will consist of three visits to our lab, which may be repeated before and following a period of exercise rehabilitation or training. The experiments on each visit day will last anywhere from two to three hours depending on the tasks being performed. A total of 270 participants will be recruited in this study.

Before agreeing to participate, please read this LETTER OF INFORMATION and ask any questions you wish.

PARTICIPANT INCLUSION/EXCLUSION CRITERIA

Overall, this investigation will study three groups of individuals: 1) healthy participants (Control Group), 2) participants with risk for cardiovascular disease, and 3) those with Coronary Artery Disease (CAD) who have recently had a cardiac event. You are invited to participate in the **Control group**.

Inclusion Criteria:
You may be included in this Control group if you are between the ages of 18 and 80 years, and if you are normally physically active and have not been diagnosed with any medical concern. Your inclusion in the Control group will be confirmed following measures of levels of glucose and triglycerides in your blood, as well as blood pressure, body size, and waist circumference. These measures will be made during your first visit (see Visit 1 below).

Exclusion Criteria:
You will not be included in the study if you smoke or have any of the following: Raynaud's disease, respiratory illnesses, diabetes, claustrophobia, history of psychosis, eating disorders, manic or bipolar disorder, major psychiatric conditions, dependence on alcohol or drugs within the past year. In addition, you will not be included in the study if you are, or think you might be, pregnant. A routine pregnancy test may be performed on women of child-bearing potential. If you are a woman of child-bearing potential you must be using an effective method of contraception.

Magnetic resonance imaging (MRI) will be used to examine the brain's vascular system in this experiment. You will not be included in this study if you have any history of head or eye injury involving metal fragments, if you have some type of implanted electrical device (such as a cardiac pacemaker). If you have severe heart disease (including susceptibility to heart rhythm abnormalities) you should not have an MRI scan

unless supervised by a physician. Additionally, you should not have an MRI scan if you have conductive implants or devices such as skin patches, body piercing or tattoos containing metallic inks because there is a risk of heating or induction of electrical currents within the metal elemental causing burns to adjacent tissue.

Finally, participants will be excluded if they are unable to provide written informed consent, or to complete questionnaires or health history forms due to language or cognitive difficulties.

STUDY DESIGN and PROCEDURE

If you agree to participate you will be assigned in a random manner (by the tossing of a coin) to one of two groups. Each group will be tested at three stages corresponding to 0, 6 and 12 months. Group 1 will begin the exercise training immediately whereas Group 2 will wait for six months before beginning the training. At each test period (i.e., 0, 6 and 12 months) you will be asked to come to the laboratory for a series of visits (see below).

Training will occur at the Laboratory for Brain and Heart Health, Room 402, Labatt Health Sciences Building. We ask you to commit to exercising at the designated site three times each week as per a program provided to you by the staff. The exercise staff include a Nurse and a Certified Exercise Physiologist. The exercise program will include approximately 30 minutes of aerobic exercise (on a bicycle ergometer or treadmill for example) and 30 minutes of strength training. The levels of exercise will be determined by a pre-study examination of your fitness level. The exercise will be progressive in the sense that as you improve, the exercise loads will increase accordingly. Your blood pressure and heart rate will be measured at each visit. Emergency equipment includes a defibrillator.

Tests: All testing and training will occur in the Laboratory for Brain and Heart Health at Western University (Room 402 Labatt Health Sciences Building).We try to schedule your testing to fit into three visits. Here is a sample of what those visits include (the order of testing may vary depending on schedules):

Visit 1 - Laboratory Testing 1 (Consent, Neurovascular Health) (3-4 hours)

Read LOI Consent Enrolment	30min supine rest	Blood Draw	Snack	Instrumentation ECG, Blood Pressure, Respiration, Waist Circumference,	Cerebrovascular Reactivity (supine 5% CO2 inhalation; Sit-to-Stand protocol).
(15min)	(30m)	(10m)	(15m)	(1 hour)	(1 hour)

Visit 2 - Laboratory Testing 2 (Vascular Imaging, Exercise Capacity, Brain blood flow) (1-2 hours)

Psychology Tests	Vascular Imaging	6-min walking test
(45 min)	(30 min)	(10 min)

Visit 3 - MRI Testing (1-2 hours)

MR Safety Screen	Instrumentation: Finger pulse oximeter Respiration, blood pressure, MoCA test	MRI brain imaging of brain blood vessels and blood flow
(15min)	(15min)	(1 hour)

Pre-Visit Preparation: We will ask that you abstain from exercise for 24 hours, and fast for 12 hours before Visit 1. Also, we ask that you abstain from exercise, and that you do not consume alcohol, nicorette gum (or any source of nicotine), coffee, tea, caffeinated soft drinks and chocolate for at least 12 hours before each Visit. At each visit, the testing will require approximately 2-4 hours of your time depending on the test sequence.

Visit 1 - Laboratory Testing 1 - Laboratory for Brain and Heart Health, HSB 402
On arrival for your first visit, you will be given opportunity to read this information letter as you decide whether or not to participate in the study. You may wish to take more time to ponder a decision about whether or not to participate in this study. If so, please feel free to take this form with you and take your time in deciding. After signing the form (and returning to the lab at the scheduled appointment), you will rest quietly for 30 minutes after which we will take a resting, fasted blood sample. A venous catheter will be inserted into a large vein near your elbow through which the research nurse (Arlene Fleischhauer) will take a blood sample. This is similar to the blood sample you would give for your annual physical, but we analyse your blood for a number of additional markers of health. We will not take more than 12 tablespoons of blood at each visit. Your blood will be analyzed for general health markers (glucose, cholesterol) as well as markers of inflammation, hormones and markers that reflect blood vessel health. One of the markers we analyze your blood for is a genetic marker called apolipoprotein (APOE). The APOE is present in 15-20% of Caucasians and has been associated with the risk of changes to brain blood vessels and cortical thickness in the brain of aging individuals.

After the blood draw is finished, we will provide you with a light snack and something to drink. You will be asked to fill in some questionnaires about your medical history while you eat and rest. You may then wish to go to the bathroom, before we measure your height and weight, and abdominal girth.

During the subsequent tests we will measure your heart rate using an electrocardiogram (ECG) and a pulse monitor attached to one of your toes. We will measure your blood pressure with a cuff around your finger, and also with a larger cuff placed around the upper part of your arm, just like it is done in a doctor's office. The arm cuff will be inflated to a high pressure for about 30 seconds to measure your blood pressure. Your rate and depth of breathing will be measured by placing a respiratory belt around your ribcage.

Brain Blood Flow Stimulation: For this task, we will have you breathe through a mask. For the first few minutes, you will breathe normal room air. You will then breathe a gas mixture that contains a higher (5%) level of carbon dioxide but the normal level of oxygen (21%) and nitrogen (74%). Carbon dioxide is a gas that your body normally produces and it increases brain blood flow. We will examine the reaction of your blood vessels and nerves to the increased level of carbon dioxide. Breathing the carbon dioxide will last up to five minutes, and will be followed by a five minute recovery period. You may be asked to breathe more frequently (in time with a rhythmic tone) for up to five minutes in order to reduce levels of carbon dioxide.

While measuring your brain blood flow, and breathing room air through this mask (or mouth piece), you will be asked to perform a series of "sit-to-stand" tasks where you will sit quietly in a chair for up to 3 minutes, stand for two minutes, and then sit again. This would be repeated up to 5 times. This task may be repeated while you are asked to either breathe a little faster or while you are breathing the 5% carbon dioxide gas mixture outlined above.

Visit 2 - Laboratory Testing 2 - Laboratory for Brain and Heart Health, HSB 402
Your second visit to the lab will involve completion of some psychological tests, characteristics of your blood vessel health, and a 6-minute walking test of your overall physical fitness.

Psychological measurements: You will undergo brief standardized testing to measure cognition, (certain features of your brain's information processing) and emotional state (mood and anxiety). This will take about 45 minutes and will consist of a series of paper-and-pencil measures and a test on the computer. In

addition, the Stroop Test is a psychological test of your mental vitality and flexibility. The task takes advantage of our ability to read words more quickly and automatically than we can name colors. If a word is printed or displayed in a color different from the color it actually names; for example, if the word "green" is written in blue ink we will say the word "green" more readily than we can name the color in which it is displayed, which in this case is "blue."

Vascular Properties: You will be asked to rest quietly on the bed as we collect 10 minutes of baseline data. The ECG and blood pressure systems outlined above for Visit 1 will be used again. Then, images of your blood vessels and blood flow will be measured using ultrasound probes placed on the skin over the arteries in your neck, arm and/or leg and brain. We will also measure the blood ejected from your heart using ultrasound. Then, we will measure the change in vessel diameter at your elbow before and for 3 minutes following, a brief (e.g., 5 minute) period where blood flow to your arm will be stopped by a cuff placed around your forearm. These ultrasound measures will be repeated before and for 4 minutes following a small dose of sodium nitroglycerine sprayed just under your tongue.

Six-minute walking test: The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in a hallway. Six minutes is a long time to walk, so you will be exerting yourself. You may become out of breath or tired. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able.

Visit 3 - MRI

The MRI visit will occur at Robarts Research Institute, 100 Perth Drive, Western University Campus.
At the beginning of your visit to the MRI we will confirm whether or not it is safe for you to enter the MRI suite by completing an MRI Safety Screen. We will put a small cuff around your finger to monitor your heart rate, and a respiratory belt around your chest to measure the depth and rate of your breathing. You will lie on a bed for about two hours while the MRI machine gathers data. MRI makes images of the interior of your body using strong magnetic and radio waves. You will not feel either. You will, however, hear loud, repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones to wear which will minimize the sound and protect your hearing.

You will rest lying down on a padded bed quietly for 10 minutes for measurement of resting heart rate. During the MRI visit we will measure the structural properties of your brain and its blood vessels while you breathe room air. Your brain's blood vessels will be measured again when you breathe the 5% carbon dioxide gas mixture outlined above for up to 5 minutes.

STUDY BENEFITS

There is the possibility that you will receive no personal benefit from this study. However, it is likely that you may be able to lower your risk for heart disease from participating. Your participation may also increase your awareness of new health habits. In reports about the study, your contributions will be grouped with those of other participants to develop conclusions that could be used to improve the education and support available for people with heart disease or who are at high risk of heart disease or stroke.

STUDY RISKS

Laboratory Test Procedures Risk

There is a small risk of bruising or infection when collecting blood from your vein. Some people may experience mild pain and discomfort and some may feel nauseous or dizzy when blood is taken. To avoid this, we will be collecting blood from you while you are lying down.

The adhesive on the electrodes used to measure your heart rate may lead to temporary redness of the skin on your chest.

There are no known harmful effects with the measures of blood vessels or blood flow using ultrasound imaging, or blood pressure, as used in this study.

The sodium nitroglycerine is a common self-administered treatment for angina pain. It may give you a mild headache for a few minutes.

Breathing a slightly higher level of carbon dioxide may give you a small headache and it may make you feel breathless. These feelings vanish quickly when you start breathing room air again.

Exercise Tests: Walking or running can be a physically challenging activity. You will breathe harder and may begin to sweat. This is part of your body's normal response to exercise. There is minimal risk associated with the self-paced six-minute walking test.

Psychology Tests
There are no risks with the psychology tests.

MRI Risk
This MRI machine uses a strong magnet and radio waves to make images of the body interior. You will be asked to lie on a long narrow couch for about 1.5 hours in each MRI session while the machine gathers data. During this time you will be exposed to magnetic fields and radio waves. You will not feel either. You will, however, hear repetitive tapping noises that arise from the magnets that surround you. You will be provided with earplugs or headphones that you will be required to wear to minimize the sound and protect your hearing. The space within the large magnet in which you lie is somewhat confined, although we have taken many steps to relieve the "claustrophobic" feeling. There are no known significant risks with this procedure at this time because the radio waves and magnetic fields at the strengths used, are thought to be without harm. The exception is if you have a cardiac pacemaker, or a metallic clip in your body (e.g., an aneurysm clip in your brain), have severe heart disease, body piercings, tattoos containing metallic ink or slow release pharmaceutical skin patches.

There is the possibility that you will experience a localized twitching sensation or perhaps a little dizzy due to the magnetic field changes during the scan. These responses are not unexpected and should not be painful. However, you can stop the exam at any time. The magnetism and radio waves do not cause harmful effects at the levels used in the MRI machine. However, because the MR scanner uses a very strong magnet that will attract metal, all metallic objects must be removed from your person before you approach the scanner. In addition, watches and credit cards should be removed as these could be damaged (these items will be watched for you).

As with any technology there is a risk of death or injury. For MRI the risk of death is less than 1 in 10 million and the risk of injury is less than 1 in 100,000. These risks do not arise from the MRI process itself, but from a failure to disclose or detect MRI incompatible objects in or around the body of the participant or the scanner room. It is therefore very important that you answer all the questions honestly and fully on the MRI screening questionnaire. Almost all the deaths and injuries related to MRI scans have occurred because the MRI operator did not know that surgically implanted metal hardware (such as a cardiac pacemaker) was present inside the participant during the MRI scan. For comparison, the risk of death in an MRI is similar to travelling 10 miles by car, while the risk of injury during an MRI is much less than the risks associated with normal daily activities for 1 hour. Any unusual findings from the MRI images will be provided to you so that you can seek further medical attention.

Exercise training Risk

All participants will obtain their family physician's signed permission to participate in exercise before they may participate in the exercise training segment.

YOUR PARTICIPATION

Voluntary Participation:

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future care, academic status, or employment. If you withdraw from the study before its completion then you may decide whether to also withdraw your data. Participation in this study will be brought to the attention of your family doctor.

The blood specimens will be discarded or destroyed once they have been used for the purposes described in the protocol. All other study data (e.g., paper files, digital files) will be kept for a minimum of 20 years.

If you are participating in another study at this time, please inform the study coordinator right away to determine if it is appropriate for you to participate in this study.

Whether you agree to participate in this study or not, you will be asked if you consent to having your name and contact information added to a master database of individuals who would be willing to be contacted in the future regarding your interest in other research studies.

Representatives of the Western University Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research. Representatives of Lawson Quality Assurance (QA) Education Program may look at study data for quality assurance purposes.

CONFIDENTIALITY

Your research records will be stored in a secure office at Western University. To further protect your confidentiality, your name will be replaced with a participant ID number on all documents. The master list linking your identity and participant ID number and your contact information will be stored separately in a secure office at Western University. Your contact information will be securely maintained at Western University to allow for setting up follow up visits. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published. No information that could reveal your identity will be released to anyone with the exception of your Family Doctor if you give permission for this.

If we find information we are required by law to disclose, we cannot guarantee confidentiality.

ALTERNATIVES TO STUDY PARTICIPATION

You may choose not to participate in this study.

REIMBURSEMENT

You will be reimbursed for travel costs up to $60 for your participation in each of the pre and post series of tests outlined above. For coronary artery disease participants, the costs associated with your participation in the cardiac rehabilitation program are provided for you. However, we cannot provide support for the travel associated with your participation in the cardiac rehabilitation program. For all participants, your parking and exercise costs will be covered for six months if you perform the exercise training in the Laboratory for Brain and Heart Health.

CONTACT PERSONS

If you have any questions about the study please contact:
IHD Research Staff: Jen Vording / Mark Badrov [redacted]

Research Nurse: Arlene Fleischhauer [redacted]
Principal Investigator: Dr. Kevin Shoemaker [redacted]

Or send an email to [redacted]
Please note that email is not considered a secure method of communication and you should not send any personal health information via email.

If you have any questions about your rights as a research participant or the conduct of the study you may contact: Dr. David Hill, Scientific Director, Lawson Health Research Institute at [redacted]

You will receive a copy of the fully signed informed consent document for your records. You do not waive any legal rights by signing the consent.

Western HealthSciences

Title: Cerebrovascular outcomes in ischemic heart disease patients undergoing cardiac rehabilitation: CONTROL GROUP
Principal Investigator: Dr. Kevin Shoemaker
Research Staff: Jen Vording, Mark Badrov & Arlene Fleischhauer

CONSENT

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

☐ I consent to be contacted for future research

SIGNATURES

_____	_____
Signature of Participant	Date
_____	
Print	
_____	_____
Signature of Person Obtaining Informed Consent	Date
_____	
Print	

Appendix 3: Raw Data Summary

Pair	%FMD		PRH		Total Shear		Baseline diameter		Relative shear		Baseline HR		Baseline SBP		Baseline DBP		Baseline MAP	
	Divers	Controls	Divers	Controls	Divers	Controls	Divers	Controls	Divers	Control	Divers	Controls	Divers	Controls	Divers	Controls	Divers	Controls
1	4.58	5.67	119.85	124.35	2998.04	2254.40	0.3710	0.4173	0.0015	0.0025	44.29	51.98	134.59	113.97	59.25	59.50	78.82	76.88
2	7.67	0.67	130.16	146.72	3064.93	5064.09	0.3520	0.4510	0.0025	0.0001	54.13	61.78	127.71	127.52	65.28	70.76	80.89	90.69
3	11.87	8.59	164.03	145.37	5062.21	3502.85	0.3987	0.4503	0.0023	0.0025	65.74	70.45	120.77	107.41	67.16	60.80	84.00	76.51
4	5.88	6.18	61.38	137.68	1771.25	3610.43	0.4590	0.4587	0.0033	0.0017	43.08	62.26	128.50	140.94	62.86	82.11	84.86	101.50
5	14.91	5.85	140.32	145.34	5526.69	5359.50	0.4517	0.4100	0.0027	0.0011	58.04	61.59	147.80	116.38	74.53	67.02	95.81	85.50
6	1.67	22.98	107.12	120.04	2737.70	3647.94	0.4180	0.4423	0.0006	0.0063	45.10	65.58	98.44	127.14	55.55	75.66	69.72	91.24
7	4.68	0.75	145.42	183.05	3731.78	5767.34	0.4127	0.4873	0.0013	0.0001	53.14	75.77	168.67	128.19	74.50	78.00	97.08	94.98
8	27.83	4.26	142.80	239.60	3751.40	4212.12	0.3473	0.2897	0.0074	0.0010	59.28	55.92	138.37	165.51	61.00	85.70	78.34	111.94
9	2.59	4.09	127.47	139.02	2342.77	2976.62	0.4377	0.5053	0.0011	0.0014	54.94	50.01	143.17	141.67	72.85	83.37	92.10	104.58
10	-2.32	14.69	140.14	160.97	5328.38	3877.04	0.5170	0.4673	-0.0004	0.0038	67.01	58.22	138.98	121.68	78.20	74.03	95.72	90.83
11	6.15	-2.59	117.22	198.75	2199.70	3012.11	0.4550	0.3347	0.0028	-0.0009	65.47	72.38	151.79	123.26	73.55	66.88	91.89	89.96
12	5.93	-3.00	132.69	104.22	4469.64	1443.12	0.4833	0.4670	0.0013	-0.0021	47.51	59.29	133.60	122.85	68.05	68.21	1.68	89.26
13	-2.83	-6.64	104.76	167.23	2252.60	3486.54	0.6123	0.5173	-0.0013	-0.0019	49.00	58.07	124.94	123.49	56.27	76.72	75.24	91.23
14	3.39	14.84	142.72	183.00	4817.33	3280.82	0.3830	0.4380	0.0007	0.0045	52.03	68.17	138.54	118.73	71.45	62.59	88.75	80.04
15	22.47	5.93	125.01	129.38	5487.58	2060.43	0.4050	0.3880	0.0041	0.0029	52.89	67.90	131.63	138.01	73.97	69.06	92.18	95.71
16	5.12	2.15	145.42	179.95	6499.22	2825.34	0.4813	0.4817	0.0008	0.0008	61.48	50.61	139.69	190.15	75.64	112.80	94.61	136.22
17	7.24	4.92	61.01	192.54	937.38	3748.58	0.3543	0.5080	0.0077	0.0013	62.05	45.60	134.83	120.95	60.74	67.60	84.15	84.06
AVERAGE	7.5	5.3	124.0	158.7	3704.62	3537.02	0.4317	0.4420	0.0023	0.0015	55.01	60.9	55.009697	60.916833	135.4141	131.04997	67.7	74.2
STD	7.7	7.0	27.1	33.3	1535.4878	1101.7838	0.0662535	0.0587398	0.0023292	0.0021144	7.49	8.2307007	23.713852	25.682871	17.462125	18.15155	21.7	14.7

Curriculum Vitae

Name:	Emilie Woehrle
Post-secondary Education and Degrees:	Western University London, Ontario, Canada BA Honours Specialization in Kinesiology, 2013-2017 Western University London, Ontario, Canada MSc in Kinesiology, Integrative Biosciences, 2017-2019
Honours and Awards:	Dean's Honour List, 2017 Western Graduate Research Scholarship, 2017-2019 Province of Ontario Graduate Scholarship, 2018-2019
Related Work Experience	*Research Assistant,* Neurovascular Research Laboratory, Western University, Summer 2017 *Teaching Assistant*, Western University, 2017-2019

Publications:

1. Moir ME, Klassen SA, Al-Khazraji BA, **Woehrle E**, Smith SO, Matushewski BJ, Kozic D, Dujic Z, Barak OF, Shoemaker JK. Impaired dynamic cerebral autoregulation in trained breath-hold divers. *J Appl Physiol. (In Press)*.
2. Schulz JM, Birmingham TB, Atkinson HF, **Woehrle E**, Lukacs MJ, Al-Khazraji BK, Primeau CA, Khan M, Zomar B, Petrella RJ, Beier F, Bryant D. A Systematic Review of the Content and Physiological Effects of Aerobic Exercise Interventions for Patients with Knee Osteoarthritis. *British Journal of Sports Medicine.* (*In Revisions)*.
3. **Woehrle E,** Harriss AB, Abbott KC, Moir ME, Fischer LK, Fraser DD & Shoemaker JK. Concussion in Adolescents Impairs Heart Rate Response to Brief Handgrip Exercise. *Clin J Sport Med. (available as ePub ahead of print)*.
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5. **Woehrle E**, Abbott KC, Harriss AB, Moir ME, Balestrini CS, Barker A, Fischer LK, Fraser DD & Shoemaker JK. Investigation of Neural Cardiac Dysregulation Using Brief 30% Isometric Handgrip Protocol in Adolescents Diagnosed with Concussion. Clin J Sport Med 27(3):35-49.

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2. Moir ME, **Woehrle E**, Smith SO, Klassen SA, Matushewski BJ, Barak OF, Dujic Z, Kozic D, Shoemaker JK. Cerebrovascular regulation in breath-hold divers with chronic exposure to long-duration apneas. *Experimental Biology*, *April 2019.*
3. Schulz JM, Birmingham TB, Al-Khazraji BK, Atkinson HF, Khan M, Lukacs M, Primeau CA, **Woehrle E**, Zomar B, Petrella RJ, Beier F, Bryant D. A systematic review and meta-analyses of the design and physiological effects of aerobic exercise for patients with knee osteoarthritis. *Osteoarthritis Research Society International, May 2019.*
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5. **Woehrle E,** Jacobs KG, Shoemaker JK. Posture modifies neuro-cardiac heart rate responses at the onset of moderate intensity isometric handgrip exercise. *LHRD, May 2018.*
6. **Woehrle E**, Jacobs KG, Shoemaker JK. Posture modifies neuro-cardiac heart rate responses at the onset of moderate intensity isometric handgrip exercise. *Experimental Biology, April 2018.*
7. Harriss AB, **Woehrle E**, Barker AL, Moir EM, Fischer LK, Fraser DD, Shoemaker JK. The impact of aerobic exercise training on autonomic function in adolescent sport-related concussion. *Experimental Biology, April 2018.*
8. Jacobs KG, **Woehrle E**, Smith SO, Klassen SA, Knetsch RJ, Barker AL, Shoemaker JK. Sex differences in heart rate response to isometric handgrip exercise with concurrent contralateral forearm somatosensory stimulation. *Experimental Biology, April 2018.*
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10. Jacobs KG, **Woehrle E**, Deck S, Kouali D, Humphreys D, Hall C, Shoemaker JK. Pilot Study: Heart Rate Variability and Mental Health Outcomes in University Female Hockey Players. *SCAPPS, October 2017.*
11. **Woehrle E,** Abbott KC, Harris AB, Moir ME, Balestrini CS, Barker AL, Fischer LK, Fraser DD, Shoemaker JK. Autonomic dysregulation in heart rate responses to brief static handgrip exercise in concussed adolescents. *Exercise is Medicine Canada National Student Conference, June 2017.*
12. **Woehrle E,** Abbott KC, Harriss AB, Moir ME, Balestrini CS, Barker AL, Fischer LK, Fraser DD & Shoemaker JK. Investigation of neural cardiac dysregulation using brief 30% isometric handgrip protocol in adolescents diagnosed with concussion. *CASEM-AQMS Sport Medicine Conference, June 2017.*
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